THE BRITISH JOURNAL OF
EXPERIMENTAL PATHOLOGY

VOL. IX JUNE, 1928 No. 3

OBSERVATIONS ON THE NATURE OF THE VAN DEN BERGH REACTION.

W. MORRELL ROBERTS.

From the Laboratory of Clinical Investigations and Research, Manchester Royal Infirmary.

Received for publication February 27th, 1928.

NUMEROUS hypotheses, most of which have been found already to be inadequate, have been advanced to explain the two types of van den Bergh reaction, the "direct" and the "indirect."

Davies and Dodds (1927) think that the two types of response may be the result of different states of oxidation of the bile-pigment in the serum, the direct reaction being due to bilirubin itself, which in hæmolytic sera has become oxidized to biliverdin or to some intermediate form. They confirmed the fact that biliverdin did not give a direct reaction, but found that, contrary to the prevailing opinion, it gave an indirect reaction.

Bilirubin is a dicarboxylic acid and so is capable of forming salts with bases. Collinson and Fowweather (1926) suggest that the indirect reaction is due to the free acid, and the direct reaction to the ammonium salt.

The experimental results about to be described show that neither of these hypotheses accounts satisfactorily for the facts.

The Oxidation Theory.

Since biliverdin gives only a greatly delayed type of reaction, the result will be indirect if the bilirubin in the serum becomes oxidized to biliverdin, but there is no doubt that this is not the explanation of the indirect response given by hæmolytic sera. This was shown by precipitating the protein from a series of hæmolytic sera (three cases of pernicious anæmia and three of acholuric jaundice) by the addition of alcohol. To the clear yellow fluid obtained by centrifugalization chloroform was added, followed by water in

excess until the chloroform separated out, containing the whole of the pigment in solution. The chloroform was shaken with very dilute caustic soda, whereupon the whole of the pigment passed into the soda, and this solution, after buffering by the addition of disodium hydrogen phosphate, gave an immediate reaction when the reagent was added. Hence the pigment concerned cannot be biliverdin, which gives a very slow reaction under these conditions. It is worth noting, further, that the sera and alcohol solutions showed the yellow of bilirubin and not the green of biliverdin, and that biliverdin is practically insoluble in chloroform.

The Chloroform Solubility and Dialyzing Properties of the Bilirubin of Jaundice Sera.

Blood-serum is so complex a physico-chemical system, and the actual mechanisms of bile-pigment formation and jaundice so incompletely understood, that it is not remarkable that sera are encountered from time to time, giving results which will not fit in with any theory yet advanced to explain the van den Bergh reaction. But, taken generally, the findings are compatible with the assumption that the bilirubin of sera giving the indirect reaction exists in the free colloidal condition, since it is not dialyzable, and is extractable from the sera by chloroform, whilst a normal serum to which colloidal bilirubin has been added behaves in a precisely similar manner. Thus, if one or two crystals of bilirubin are dissolved in a drop of chloroform, a few drops of alcohol added and the solution poured into water, a faintly opalescent colloidal solution of bilirubin results, which gives a greatly delayed reaction, and shows no visible change after several weeks' standing. If some of this colloidal solution is added to normal serum a similar type of reaction is obtained. When alcohol is added to the serum the reaction is practically instantaneous. The bilirubin will not dialyze, but may be extracted from the serum by chloroform.

The pigment of obstructive sera, on the other hand, will not extract to any great extent with chloroform. It has also been found by most workers to be dialyzable, and this was verified in the course of the present work. Further, it may be dialyzed from an obstructive serum to a normal serum, whereupon the latter shows a similar but rather more rapid type of reaction than that given by the obstructive serum.

Davies and Dodds do not consider that the chloroform-solubility properties of bilirubin can be brought forward to distinguish between obstructive and hæmolytic bilirubin. But the fact that there is a general tendency for the latter to be the more soluble, and that this is not due entirely to the physical properties of the respective sera, may be demonstrated as follows: The proteins are precipitated from 2 c.c. of serum by the addition of 4 c.c. of alcohol and the mixture centrifuged. To 3 c.c. of the clear liquid 2 c.c. of chloroform are added, followed by 5 c.c. of water added 1 c.c. at a time, the mixture being shaken and centrifuged after each addition, and an estimate made of the relative amounts of colour in the two layers, *i.e.* the upper alcohol-water layer and the lower chloroform layer. The type of result obtained is shown in the following table, though the findings are not always as clear-cut as these:

	Obstructive serum.	Hæmolytic serum.
After addition of first c.c. of water	Colour all in upper layer	Most colour in upper layer. Trace in lower layer.
Ditto second c.c. water	Ditto	Colour approximately equal in both layers.
Ditto third c.c. water	Ditto	? All colour in lower layer.
Ditto fourth c.c. water	? Trace colour in lower layer	All colour in lower layer.
Ditto fifth c.c. water	Trace colour in lower layer	Ditto.

The Ammonium Salt Theory.

If Collinson and Fowweather's suggestion is correct there are certain consequences that would be expected to follow:

1. *The addition of hydrochloric acid to obstructive sera should render the pigment soluble in chloroform, and the addition of ammonia to hæmolytic sera should prevent its extraction.*

After the addition of varying proportions of hydrochloric acid to obstructive sera it was still found to be impossible to extract the bilirubin. Similarly, sera to which colloidal bilirubin or a solution of sodium or ammonium bilirubinate had been added, followed by hydrochloric acid, failed to extract. On precipitation of the serum protein by alcohol a reaction was obtained at once in all these cases. The addition of ammonia to hæmolytic sera rendered the pigment insoluble, but so, too, did the addition of acid. On the addition of the exact amount of alkali necessary to neutralize the added acid, the pigment could again be extracted by chloroform. It appears as if the addition of acid, and possibly also of ammonia, alters the properties of the serum so that the free bilirubin is adsorbed by the proteins and therefore will not extract.

2. *The addition of acid to obstructive sera should delay the reaction, and the addition of ammonia or soda to hæmolytic sera should accelerate it.*

Small proportions of acid added to obstructive sera caused no measurable delay (*e.g.* 0·1 c.c. serum + 0·05 c.c. decinormal hydrochloric acid). Definite delay occurred only when sufficient acid was added to lower the pH to near the point below which the reaction cannot occur.

The apparent accelerating effect of dilute ammonia on a hæmolytic serum may be due wholly or in part to the colour which the reagent gives with ammonia alone. After the addition of sufficient ammonia to bring about this effect, followed by the reagent, a colour appears at once, viz., an orange tint which gradually becomes red-violet. But a control with ammonia + normal serum and reagent shows a similar initial colour development, which, of course, does not progress to the red-violet of the delayed reaction.

3. *The addition of ammonium bilirubinate to normal serum should lead to a direct reaction.*

Since bilirubin is a very weak acid, its salts are hydrolytically dissociated in water, and it is necessary to have an excess of ammonia present to prevent the dissociation. In practice, sufficient ammonia was added so that no bilirubin was extracted from the solution on shaking with chloroform. When a little of such a solution was added to normal serum it was possible to obtain only a greatly delayed reaction. When solid ammonium bilirubinate (prepared by salting out by ammonium chloride) was added to serum, it was hydrolytically dissociated, with the result that the bilirubin extracted with chloroform, and the serum again showed a delayed reaction. The sodium and potassium salts behaved in an exactly similar manner to the ammonium salt.

These facts do not support the hypothesis of Collinson and Fowweather, against which there is further evidence.

If bilirubin is dissolved in sufficient excess of ammonia to prevent any extraction on shaking with chloroform, the solution buffered by the addition of disodium hydrogen phosphate, and carbon dioxide passed through for a few minutes, the whole of the bilirubin is set free and may be extracted by chloroform.

When such a solution of the ammonium salt is added to normal serum and the procedure already described for observing the relative solubility of the pigment in alcohol-water and in chloroform carried out (in this case the whole 5 c.c. of water is added at once), the pigment remains in the alcohol-water layer until carbon dioxide is passed through the latter, whereupon the pigment dissolves entirely in the chloroform on shaking. When a rapid biphasic serum (diluted with water to the same degree as the normal serum is diluted with ammonium salt solution) is treated in the same way, essentially similar results follow.

If, however, the solution of ammonium salt plus serum is treated with carbon dioxide *before* the relative solubility procedure is carried out, the bilirubin appears entirely in the chloroform layer at once, whereas if the prompt biphasic serum is similarly treated the pigment is still found mainly in the alcohol-water layer until this is treated with carbon dioxide, when it passes as before to the chloroform layer.

The sodium and potassium salts of bilirubin behave like the ammonium salt under these conditions. Presumably also a direct serum would show similar results to a prompt biphasic serum, but a *true* direct serum, *i. e.*, one giving a blue-violet colour with the van den Bergh reagent maximal in thirty seconds, has not yet been met with in this hospital.

The above results are summarized in the table, and suggest that although the bilirubin of obstructive sera may exist as the ammonium salt *after* the addition of alcohol, it does not exist in this form *before* the addition. But if it is not present as a salt until after alcohol has been added there is no longer any advantage in assuming that it is in the form of the ammonium rather than of the sodium or potassium salt; in fact, owing to the presence of the buffer substances sodium bicarbonate and disodium hydrogen phosphate and the small amount of ammonium in the serum, it is most likely to be present as the sodium salt.

	Treatment.	Result.	Further treatment.	Result.
1. Solution of ammonium bilirubinate with excess ammonia and buffered with Na_2HPO_4	CO_2 passed through	Bilirubin liberated and completely extractable by chloroform	...	...
2*a*. Solution of ammonium bilirubinate with excess ammonia, buffered with Na_2HPO_4 and added to normal serum	Relative solubility procedure carried out	Bilirubin found in alcohol-water layer; chloroform layer colourless	CO_2 passed through alcohol-water layer	Bilirubin passes to chloroform layer on shaking.
2*b*. Prompt biphasic serum	Ditto	Pigment found chiefly in alcohol-water layer; chloroform layer almost colourless	Ditto	All pigment passes to chloroform layer on shaking.
3*a*. Solution of ammonium bilirubinate with excess ammonia, buffered with Na_2HPO_4 and added to normal serum. (Same solution as in 2*a*)	CO_2 passed through mixture; relative solubility procedure then carried out	Bilirubin found in chloroform layer; alcohol-water layer colourless	...	...
3*b*. Prompt biphasic serum. (Same serum as in 2*b*)	Serum treated with CO_2; relative solubility procedure then carried out	Pigment found chiefly in alcohol-water layer; chloroform layer almost colourless	CO_2 passed through alcohol-water layer	All pigment passes to chloroform layer on shaking.

SUMMARY.

1. The pigment of hæmolytic sera gives a direct reaction if extracted by chloroform and dissolved in an alkaline buffered solution. This is in opposition to the hypothesis of Davies and Dodds that the pigment of hæmolytic sera is present as an oxidation product of bilirubin.

2. Evidence is adduced in favour of the fact that bilirubin in such sera is present largely in the free colloidal condition.

3. The suggestion of Collinson and Fowweather, that in those sera giving the direct reaction the bilirubin is present as the ammonium salt, is shown to be untenable. It appears that the bilirubin is in combination with some substance, the nature of which is as yet undetermined, this combination being destroyed by the addition of alcohol, leaving the bilirubin as a salt—probably the sodium salt.

The subject is under further investigation, particularly the problem of the form in which the bilirubin exists in obstructive sera prior to the addition of alcohol.

I am indebted to Prof. Craven Moore and Prof. Raper for the interest they have taken in this work.

REFERENCES.

COLLINSON, G. A., AND FOWWEATHER, F. S.—(1926) *Brit. Med. J.*, **1**, 1081.
DAVIES, D. T., AND DODDS, E. C.—(1927) *Brit. J. Exper. Pathol.*, **8**, 316.

BILIRUBIN AND THE VAN DEN BERGH REACTION.

C. E. NEWMAN.

From King's College Hospital, London.

Received for publication April 3rd, 1928.

A GREAT deal of work has been done during the last few years on the chemistry of the van den Bergh reaction, and very divergent hypotheses put forward. These have already been ably reviewed (Davies and Dodds, 1927), so I shall simply put forward some new experimental observations, and show how they reconcile several apparently inconsistent solutions. The matter will be dealt with in three stages: (*a*) a confirmation and amplification of the observations on pH made by Davies and Dodds; (*b*) some investigations on biphasic sera; and (*c*) a summary of the properties of bilirubin and a suggested explanation.

(*a*) *Azobilirubin as an Indicator.*

Many observers have found, as we did, that the colour of the indirect van den Bergh test is often slightly more blue than the colorimetric standards.

This difference makes accurate estimation extremely difficult or impossible, and as quite a large percentage of "prompt reaction" sera show it, the matter is of some practical importance. We found that any completed van den Bergh reaction could be made slightly blue by the addition of a trace of acid—in fact that azobilirubin behaves to acids and alkalies as an indicator (Thannhauser and Andersen, 1921). The colour of the typical van den Bergh reaction is best described as rose pink, being pink with a slightly blue tint. If a drop of concentrated hydrochloric acid is added to any such solution the colour is changed to an indigo blue. If, now, dilute soda is added drop by drop, the colour changes through purple and blue-pink to the typical rose, and then through pale red and greenish grey to olive green in extreme alkalinity. The exact pH values at the different stages of this process were worked out by comparison with other indicators, and the results controlled electrically by the kindness of Prof. Dodds and Mr. Maclagan, of the Bland Sutton Institute. The solution is indigo to a pH of about 1·0, rose at pH 2·2, and clear bright red at pH 5·5. The final change to olive green takes place outside the ordinary pH range in an excess of alkali.

The same colour changes are produced if the original bilirubin-containing solution is treated with suitable quantities of alkali before diazotization, but in this case it is also noticed that the rate of reaction changes with the pH, being prompt down to somewhere between pH 2 and 3 (probably pH 2·8, the figure given by Davies and Dodds), and below that delayed. The above observers do not note that the reaction does eventually take place. The practical difficulty of the colour difference between the unknown and the standard in the quantitative estimation may be overcome by using as the diluting fluid alcohol containing 10% of concentrated hydrochloric acid instead of pure alcohol. This turns the solution indigo, and it is matched against an indigo-coloured standard. The standard I am using is a solution of aniline gentian violet in alcohol, made up to match an acidified 1 unit van den Bergh test, but it is not altogether satisfactory.

Effect of Acids and Alkalies on Sera.

The addition of any of the sodium alkalies in proper amounts to delayed reaction sera will convert the reaction to a prompt one. This may be demonstrated as follows: ½ c.c. of delayed reaction serum is put into each of a series of small test-tubes. A dilute soda solution is prepared by making 3 c.c. of normal sodium hydroxide up to 100 c.c. with distilled water. One drop of this is added to the first tube, two to the second, and so on. ½ c.c. of diazo reagent is then added to each tube, and in one of them (the one containing 4 drops of alkali in one of my series, for instance) a perfect prompt reaction will be obtained. The addition of acid will similarly delay the reaction of prompt sera. (Sulphuric or hydrochloric acids may be used; nitric acid oxidizes bilirubin to biliverdin.)

Effect of Ammonia on Sera.

Sodium, potassium or barium alkalies all produce much the same results, but sodium reproduces the appearance of natural serum reactions more closely

than the others. Ammonia, which was brought into prominence by Collinson and Fowweather (1926), gives entirely different results. In the first place, the addition of ammonia to the diazo reagent alone turns it bright yellow, and this gives a spurious appearance of promptness to reactions. If a series of tubes, similar to that described above, is put up, with ammonia instead of soda as the alkali, it will be found that they all give an instantaneous brown colour when the diazo solution is added. Exactly the same colour is seen if a tube of diazo solution plus ammonia is viewed behind a tube of serum in a comparator. Secondly, if a solution of azobilirubin is made strongly alkaline with ammonia, a very remarkable pigment is formed. The solution is grey-green by reflected light, but seen down the length of the tube, or by transmitted light in strong solution, is a rich wine red. Moreover, this solution, unlike that made with soda from natural sera or artificial bilirubin solutions, cannot be made rose or blue by the addition of acids, but only a pale red colour. These observations make the ammonium bilirubin theory of Collinson and Fowweather most unlikely as an explanation of the prompt and delayed reactions.

(*b*) *On Biphasic Sera.*

On the supposition that biphasic sera give this type of reaction because both kinds of bilirubin are present, they have been a favourite subject for investigation. True biphasic sera, that is, those giving what used to be called the "biphasic delayed" reaction, are very uncommon, but there is fortunately a plentiful source of material in the ascitic fluids of patients suffering from obstructive jaundice, which usually give perfect biphasic reactions.

Synthesis of Biphasic Sera.

An artificial biphasic serum may be made by putting up a series of ten tubes, containing progressively increasing amounts of a strong delayed reaction serum (about 3-unit strength) from 0 to 10 drops, and progressively decreasing amounts of a weak prompt reaction serum (about 1 unit strength) from 10 to 0 drops. One of the tubes (the one containing 8 drops of "delayed" and 2 drops of "prompt" in one of my series, for instance) will give a perfect biphasic reaction.

Analysis of Natural Biphasic Sera.

The bilirubin in prompt reaction sera is soluble in water, but not in chloroform, whereas that in delayed reaction sera is soluble in chloroform. If a biphasic serum is shaken with chloroform, a good deal of pigment is extracted, and after 2 or 3 washings the chloroform extracts no more. The watery residue now gives a perfect prompt reaction. That the pigment extracted by the chloroform is bilirubin can be shown as follows: Evaporate the chloroform washings to a volume of about 0·5 c.c. on a water-bath, add 4 drops of diazo solution, and then add alcohol till the chloroform and the watery diazo reagent both go into solution in the alcohol, when a van den Bergh reaction will take place. If a serum can be obtained which contains very little bilirubin, or none at all, the chloroform may be evaporated to dryness, and the dried delayed

reaction pigment dissolved in this serum, which will then be found to give a perfect delayed reaction.

Biphasic sera, therefore, unquestionably contain the pigments of both prompt and delayed reaction sera, both existing and maintaining their characteristics in the same serum simultaneously, and the biphasic reaction is, as has been supposed, a weak prompt reaction reinforced after a time by a delayed reaction. This at once disproves the first theory put forward by Davies and Dodds, that the nature of the reaction depends on the medium in which the bilirubin is dissolved, as the medium is here the same for both, and yet they maintain their differences.

(*c*) *Table of Reactions.*

The reactions of various bilirubin-containing substances may here be set out in tabular form to save space. My so-called "pure bilirubin" was made by shaking bile, acidified with HCl, with chloroform, pipetting off the pigment-containing chloroform, and evaporating it to dryness, the whole process being performed in a stream of coal-gas to keep away the oxygen, which otherwise oxidizes the bilirubin at once to biliverdin. Needless to say, the product is not pure, but the results obtained with it correspond exactly to those obtained by workers who were fortunate enough to have real pure bilirubin.

The main points to note are: (1) Acidification delays all reactions, and makes the pigments soluble in chloroform. (2) Alkalies have the opposite effect. (3) *Delayed reaction pigment is not the same as pure bilirubin.* This is of the utmost importance, as the assumption that they are identical has been a veritable nest-egg of confusion in the past. It will therefore be as well to give the reasons for this statement:

(*a*) Pure bilirubin dissolved in normal serum gives a prompt van den Bergh reaction, whereas the pigment extracted from a serum giving the delayed reaction, redissolved in serum, gives a delayed reaction.

(*b*) Delayed reaction bilirubin in alcoholic solution, after standing a week in air, still gives a van den Bergh reaction instantaneously and can be extracted by chloroform. In other words it is unchanged. Pure bilirubin, on the other hand, at the end of a week under precisely the same conditions is completely oxidized to biliverdin, which is green in colour, insoluble in chloroform,* and does not give an instantaneous van den Bergh reaction in alcoholic solution.

(*c*) Delayed reaction bilirubin in a buffer of pH 7·3 for a week in air is unchanged, whereas pure bilirubin under the same conditions is oxidized to biliverdin.

(*d*) Both pigments may be changed to prompt reaction bilirubin by the addition of soda, but a greater concentration of soda is needed to act on delayed reaction bilirubin than is needed for the chemically prepared bilirubin. For instance, if delayed reaction bilirubin is dissolved in 2 c.c. of water containing 0·002 gm. of NaOH, it still gives a delayed reaction, whereas pure bilirubin in the same solvent gives a prompt reaction. This is the explanation of the curious fact noted by Andrewes (1924) concerning delayed reaction bilirubin in bile. He observed that if bile is shaken with chloroform some pigment is

* This is sodium biliverdinate. Biliverdin in acid solution is soluble in chloroform.

Material.	Reaction with diazo solution alone.	Reaction with diazo solution after acidification.	Reaction with azo solution after alkalization.	Adsorption of pigment by the protein precipitate in the indirect test.	Solubility of pigment in chloroform.	Solubility of pigment in water.	Rate of oxidation of pigment in air (see text).	Ease of conversion to "prompt reaction" pigment by alkali (see text).
Sera of obstructive jaundice	Prompt; rose colour	Delayed; blue colour	Prompt; red colour	Much adsorption	Insoluble (except for the trace of physiological pigment present in all sera)	Soluble	In about 1 to 14 days	...
Sera of hæmolytic jaundice	Delayed; rose colour	Delayed; blue colour	Prompt; red colour	No adsorption	Soluble (completely extractable)	Insoluble, except as a colloidal solution	Very slow; weeks or months, sometimes apparently never	Needs a fair concentration of alkali.
Biphasic sera	Biphasic (red in 5 to 15 secs., deepening to rose in half-an-hour)	Delayed; blue colour	Prompt; red colour	Little adsorption	Some pigment extractable, some left in watery layer	Part soluble	Partly oxidized in about a week	...
Bile. Diluted 1:10	Prompt (usually); purple colour	Delayed; blue colour	Prompt; red colour	Much adsorption	Most of the pigment insoluble. A little delayed reaction pigment usually present	Most of the pigment soluble	Variable; minutes to months	...
Pure bilirubin	In water: Delayed; blue colour. In alcohol: Prompt; blue colour	In water: Delayed; blue colour. In alcohol: Prompt; blue colour	In water or alcohol: Prompt; red colour	If dissolved in serum: Much adsorption	Freely soluble	Insoluble	Extremely rapid; a few shakes in air (in alcohol)	Needs only an exceedingly low concentration of alkali.

extracted, which, when re-dissolved in distilled water containing a trace of alkali, gives a delayed reaction. This pigment is delayed reaction bilirubin, which is present in varying amounts in all specimens of bile, and is probably formed from the prompt reaction pigment by being kept in the gall-bladder (*vide infra*). Simple spontaneous conversion also probably explains the biphasic reaction of ascitic fluid in obstructive jaundice.

The distinction between delayed reaction bilirubin and pure bilirubin will be found to reconcile most of the apparent inconsistencies in the explanations put forward of the van den Bergh reaction. The great point is that the slow development of colour produced by lowering the pH is not given by delayed reaction bilirubin but by pure bilirubin. There is no need to stress the point here, as it will be much clearer in the discussion of results.

The Conversion of Prompt to Delayed Reaction Bilirubin.

It was suggested by Dr. MaCance that the reason a prompt reaction serum deteriorated on keeping was that the pigment was changed to delayed reaction bilirubin. To investigate this the serum from a case of obstructive jaundice, which gave a prompt reaction and in which none of the pigment was soluble in chloroform, was put up in a series of tubes in an oxygen-free atmosphere of hydrogen, and left to stand. The serum was put up on 19/9/27. On 28/9/27 a sample still gave a prompt reaction. On 15/11/27 it gave a biphasic reaction, and nearly two-thirds of the total pigment was soluble in chloroform. The last sample, on 10/1/28, gave a faint trace of biphasic reaction, but nearly all the pigment was in the form of chloroform-soluble delayed reaction bilirubin.

A solution of pure bilirubin in serum was also put up under identical conditions on 23/9/27. On that day it gave a prompt reaction and contained 4 units of bilirubin. On 10/1/28 it gave a biphasic reaction in which there was only a trace of the prompt phase, and 2·9 units of delayed reaction bilirubin were extracted with chloroform—that is, nearly three-quarters of the pigment had been converted.

This reaction, which is now being worked out in detail by Dr. MaCance, completes the cycle of *in vitro* conversions of one type of pigment into another. Prompt and delayed can be converted to pure bilirubin by acidification. Pure bilirubin and delayed can be converted into prompt by the addition of soda. Pure bilirubin and prompt can be converted to delayed by leaving them to stand in solution in serum.

Discussion of Results.

I have dealt with the experimental work as briefly as possible to avoid repeating what Davies and Dodds (1927) have already published. Their paper should be read in conjunction with this. I am afraid that in the interpretation of results my views are rather different. The evidence seems to indicate that the different van den Bergh reactions are due to an underlying chemical difference between the bilirubins. The behaviour of azobilirubin as a dichromic indicator and the many differences between the bilirubins from different sources shown in the table, together with the constitutional formulæ suggested by Küster and others, all point to its being, as pure bilirubin, an

organic acid containing two COOH groups and capable of forming two series of salts. The differences between pure bilirubin, the acid, and the delayed reaction pigment seem to indicate that the latter is a substance closely allied to the former, but considerably more stable. Pure bilirubin could not circulate as such in the blood, since it is changed on solution in serum to the prompt reaction type of pigment. I hope to deal with the chemical nature of delayed reaction bilirubin in a future paper.

We are on much firmer ground in suggesting that prompt reaction bilirubin is sodium-hydrogen-bilirubinate. The di-sodium salt exists only in extreme alkalinity, and does not concern physiology or pathology.

The experimental work of Davies and Dodds can be explained on these lines. Their buffer solution was a weak alkali, and gave results which would have been expected from it as such. Pure bilirubin in such a solution is converted to the prompt reaction pigment. The theory that the delayed reaction pigment is biliverdin or some intermediate oxidation product can be narrowed down. Biliverdin it cannot be, because this substance is green in colour, insoluble in chloroform, and only gives the van den Bergh reaction in 12 to 24 hours—all points of distinction from the delayed reaction pigment. Intermediate oxidation products do not seem very likely, because of the extreme resistance of the delayed reaction pigment to oxidation, and I can find experimentally no stage in the oxidation of bilirubin where further oxidation becomes difficult and the pigment ceases to be soluble in chloroform. Their discovery that biliverdin does give a van den Bergh reaction I can confirm.

SUMMARY.

1. Azobilirubin is an indicator, with a double colour change.

2. Biphasic sera can be analysed into, and synthesized from, pigments giving prompt and delayed reactions.

3. Pure bilirubin is not the same substance as the pigment giving the delayed reaction in nature.

4. There are four chemically distinct bilirubins, two occurring in nature and two in the laboratory. Their differences may be summarized as follows:

A. *Free Acid Bilirubin.*

Made by acidifying B, C or D.
Soluble in chloroform, but not in water.
Gives the delayed reaction, blue colour.
Easily converted into salts or oxidized.

B. *Delayed Reaction Bilirubin.*

In normal and hæmolytic jaundiced sera.
Can be made from A or C by standing in solution in serum, in an oxygen-free atmosphere.
Soluble in chloroform, and as a colloidal solution in water.
Gives a delayed reaction, rose colour.

C. *Sodium Hydrogen Bilirubinate.*

In obstructive jaundiced sera, and bile.
Soluble in water, but not in chloroform.
Gives a prompt reaction, rose colour.
Converted to A by acids, to B by standing in serum.

D. *Di-sodium Bilirubinate.*

Does not exist in nature.
Made from A, B or C by excess of soda.
Soluble in water, but not in chloroform.

My thanks are due to Dr. Creed and Dr. Lawrence for much helpful advice and criticism, and for permission to do this work. Also to Dr. MaCance for his great assistance, and to Dr. McNee, Prof. Dodds and Dr. Davies for their great kindness and courtesy, and to Mr. N. Maclagan of the Bland Sutton Institute for his pH determinations.

REFERENCES.

ANDREWES, C. H.—(1924) *Brit. J. Exper. Pathol.*, **5**, 213.
COLLINSON, G. A., AND FOWWEATHER, F. S.—(1926) *Brit. Med. J.*, **1**, 1081.
DAVIES, D. T., AND DODDS, E. C.—(1927) *Brit. J. Exper. Pathol.*, **8**, 316.
THANNHAUSER, S. J., AND ANDERSEN, E.—(1921) *Deutsch. Arch. f. klin. Med.*, **137**, 179.

SOME OBSERVATIONS ON THE EFFECTS OF ETHER ANÆSTHESIA ON THE COMPOSITION OF THE BLOOD AND THEIR RELATION TO THYROID FUNCTION.

H. E. MAGEE, W. ANDERSON AND AGNES E. GLENNIE.

From the Rowett Institute, Aberdeen.

Received for publication April 11th 1928.

THE objective of the experiments recorded in this paper was to supplement the data already obtained showing some effects of ether anæsthesia on the composition of the blood (Magee and Glennie, 1927). As these seemed to indicate a possible connection with thyroid function, the influence of ether anæsthesia on thyroid-fed and thyroidectomized animals was also investigated.

Rabbits were used for all the experiments. Those that were fed on thyroid received daily doses of 0·5, 1 or 4 gm. of dried gland, according to whether the effect of small, medium or large doses was under consideration. The animals varied considerably in the readiness with which they took their ration.

Thyroidectomy was done under ether with aseptic precautions. A week generally sufficed for complete healing of the operation wound, during which time no samples of blood were drawn.

Blood was drawn from the ear, the rabbits having fasted for 15 hours previously. In a very few cases when the animals were in poor condition they were stunned, the chest was opened and the blood drawn from the still beating heart.

The methods employed have been mentioned (Magee and Glennie, 1927). Iodine was determined by the method described by Leitch and Henderson (1926). In the first place the changes in the blood due to the experimental hyper- and hypo-thyroidism alone will be described, and then those due to anæsthesia on normal, thyroid-fed and thyroidectomized animals.

Effects of Thyroid Feeding and Thyroidectomy.

In Table II some of the pH, sugar and P findings are shown. Those for Ca and iodine are recorded separately.

Normal animals.—The Martin-Lepper method (1926) for estimation of pH we found to be very satisfactory if all the precautions mentioned were taken. The mean of 40 readings in triplicate on 30 animals was 7·30, with variations from 7·25 to 7·35. The great majority, however, came very close to the mean. A sluggish flow of blood generally resulted in low readings. This could be overcome by keeping the ear very warm. In drawing the blood the small artery as well as the vein was usually severed, so that the results may be taken to refer to mixed blood.

A significant number of the inorganic P readings was lower than the others. The former values were all obtained during April and May, while the others were obtained during July, September and October. Table I, showing the average values obtained in this laboratory at different times, suggests a seasonal variation in blood P. The number of animals experimented on varied from 3 to 10 in each period shown.

TABLE I.

Month:	Jan.	March.	April.	May.	June-July.	Sept.	Oct.	Nov.	Dec.
Mg. P per 100 c.c.	5·18	3·04	3·22	4·5	7·00	6·51	6·58	6·93	6·17

These results support those of Hess and Lundagen (1922).

Thyroid-fed animals.—Fifteen blood analyses were done on 9 animals after varying doses of thyroid. The pH did not show any definite change until very large doses were fed and then it was appreciably lowered (*e.g.* No. 14). At this stage the animals were obviously ill. Sugar rose at first and then fell as the dose was increased (*e.g.* No. 40). Bodansky (1924), Burn and Marks (1925) and Hancher *et al.* (1925) have found similar results. Such a finding was not unexpected; for the accelerating effect of thyroxine in excess on glycogenolysis would cause a flow of glucose into the blood-stream so long as the store of glycogen in the liver was adequate. When it failed some degree of hypoglycæmia would result. The inorganic P was greatly increased by large, but unaffected by small doses of gland. This finding suggests disorganization

of phosphorus metabolism, which may have been related to depletion of the glycogen reserves.

Thyroidectomized animals.—Twenty blood analyses were done on 12 animals at varying intervals after operation. The results showed widely varying changes in sugar, P and pH. Seven of the sugar values were low, ranging from 17 to 111 mgm. They were accompanied by increases in P and falls in pH, except in one. The remaining sugar values varied from 114 to 218. These were associated with raised P values in 8 cases and with unchanged or lowered values in 5 cases. The accompanying pH values showed no effect or a slight fall. It may be that these two sets of sugar values indicate increasing degrees of compensation for the lost glycogenolytic function of the thyroid. The rises in the sugar shown by 4 animals (*e.g.* No. 33) as the post-operation time increased would seem to support this view; but estimations of liver glycogen would have been necessary to prove this point. The significance of the varied changes in pH and P is not apparent.

Calcium.—It was not possible, except in a few cases, to determine the blood Ca before and after thyroid feeding or before and after thyroidectomy in the same animals. Average figures, therefore, are given only. Seventeen estimations on 14 normal animals gave a mean value of 13·9 mgm. Ca per 100 c.c. serum with variations from 12·5 to 15·5 mg. Five estimations on 5 animals fed 10 to 16·5 grm. of thyroid gave an average of 15·2 mgm., with variations of 14·1 to 16·1 mgm. Five readings on 4 animals thyroidectomized 15 to 26 days previously gave a mean of 12·8 mgm. with variations of 11·7 to 14·3. These results would suggest that thyroid feeding increases the blood Ca and thyroidectomy lowers it. This view is supported by the experiments of Rabinowitch (1924) and Kunde (1925).

Iodine.—These results were so very definite that their expression as mean values was completely justifiable. Eight normal animals had an average blood iodine of 9·4γ per 100 c.c. with variations of 8·6 to 9·8γ. The same animals after being fed with 10 to 31 gm. of thyroid gave an average iodine content of 63·9γ with variations of 38 to 116γ. There was no clear relationship between dosage and iodine content. Five animals thyroidectomized from 9 to 19 days previously showed a mean value of 5γ, with variations of 4 to 6γ. Their blood iodine was not determined before operation, but as the normal values were so consistent and as all the iodine estimations were done at the same season, there can be no doubt but that the big drop was due to thyroidectomy. Orr and Leitch (1928) in this Institute also obtained very low values in thyroidectomized animals. The iodine content of the blood seems, therefore, to be a criterion of thyroid activity.

TABLE II.

	Normal.			*Thyroid-fed.*			
Rabbit No.	pH.	Sugar mgm. %.	P. mgm. %.	Gm. thyroid.	pH.	Sugar mgm. %.	P. mgm. %.
40	...	143	5·81	1·0	...	153	5·77
40	...	143	5·81	3·0	7·28	247	5·16
40	...	143	5·81	11·0	7·28	132	6·86
14	7·25	191	3·53	28	7·18	159	8·36

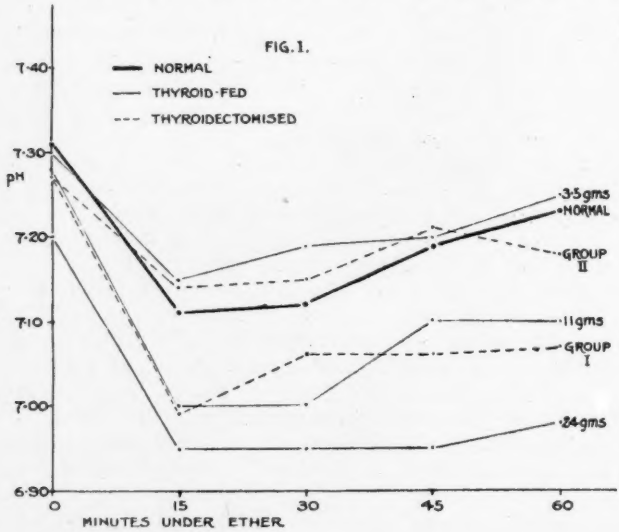
TABLE II—*continued.*

	Normal.			*Thyroidectomized.*			
Rabbit No.	pH.	Sugar mgm. %.	P. mgm. %.	Days after operation.	pH.	Sugar mgm. %.	P. mgm. %.
28	7·30	154	4·39	8	7·25	95	7·15
33	7·33	119	8·26	7	7·30	154	4·06
33	7·33	119	8·26	16	7·34	218	6·09
47	7·29	140	6·54	11	7·30	125	7·44

Effects of Anæsthesia.

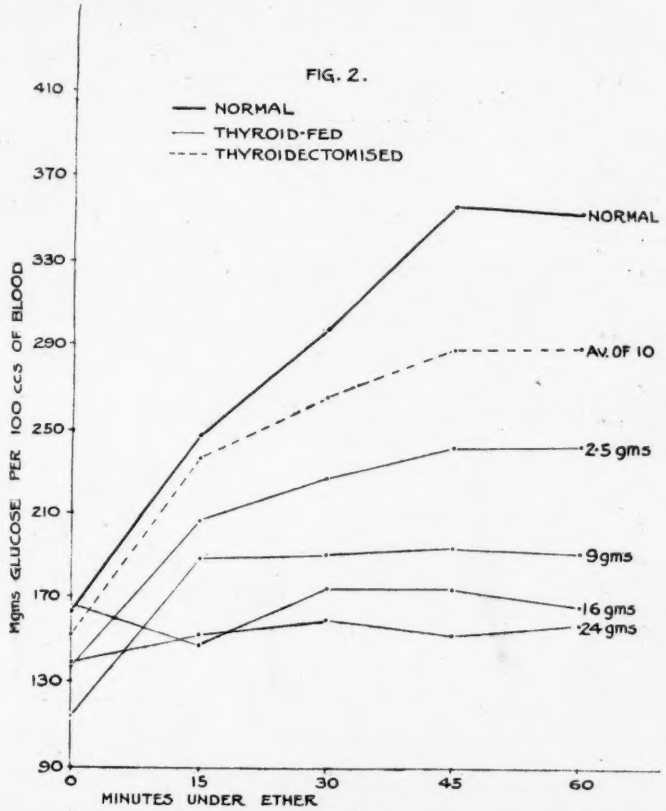
Figs. 1, 2 and 3 show the pH, sugar and P findings. Those for Ca and iodine are recorded separately.

Normal animals.—Thirty-five experiments were performed on 30 animals. The pH, sugar and inorganic P were determined every 15 minutes up to an hour's anæsthesia in 10 of these experiments. All the results obtained were of similar nature to those already recorded (Magee and Glennie, 1927), but the P rose definitely in every case except one. The values referring to the 10 complete experiments were therefore averaged and plotted (Figs. 1, 2 and 3).



Thyroid-fed animals.—Twelve complete experiments were performed. The pH and P values were variable and representative curves only are shown. Small doses (3·5 gm.) had no appreciable effect on the general shape of the pH curve. After medium doses (11 gm.) a more pronounced acidosis occurred and recovery was less complete than normal. After large doses (24 gm.) acidosis was still more pronounced and there was scarcely any

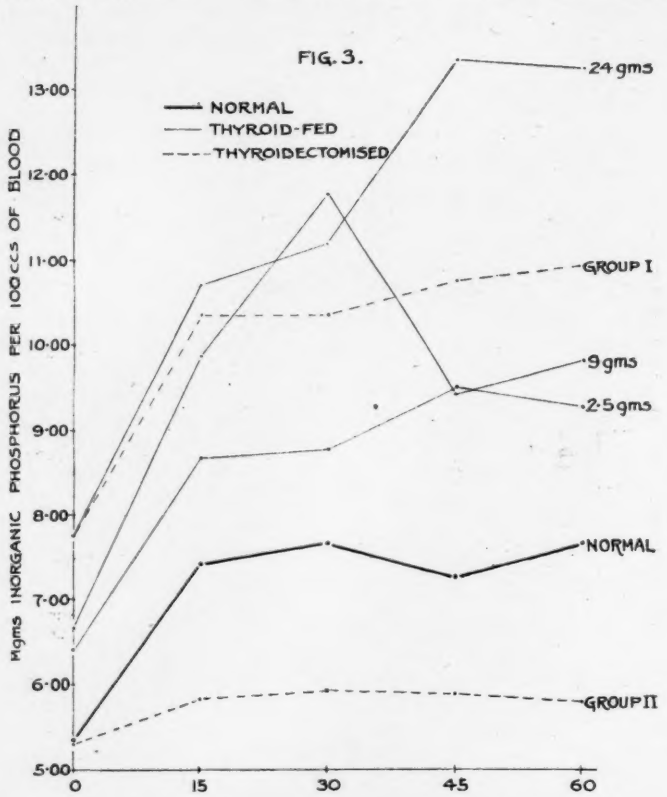
recovery (Fig. 1). With large doses (24 gm.) the P rose fairly steadily to very high values. After small doses the P was generally at a maximum after 30 or 45 minutes' anæsthesia and fell towards the end, but never to the pre-anæsthesia level. Even small doses gave curves at a higher level than normal. There was, however, no hard and fast relation between the dosage and the type of curve (Fig. 3). Thyroid feeding caused progressive lowering of the hyperglycæmic response to ether (Fig. 2).



Thyroidectomized animals.—Fifteen animals survived long enough to permit of further experiments, but 10 complete experiments only were obtained, 3 having died early during anæsthesia. The pH and P values fell into 2 distinct groups of 5 each, which were respectively averaged and plotted (Figs. 1 and 3). In both groups the pre-anæsthesia pH values were identical, 7·27, but the P value was 7·76 in Group 1 and 5·30 in Group 2. The animals in Group 1 showed, under ether, a distinct increase in P above normal and marked and prolonged acidosis. In Group 2 the P arose

scarcely at all and only moderate acidosis occurred. No close relationship was distinguishable between the pH or P and the sugar curves in the individual experiments. All the sugar values, irrespective of the pre-anæsthesia level, showed a lower hyperglycæmic response to ether than normal. An average of them all was therefore taken (Fig. 2).

Calcium.—Subsequent experiments on normal animals confirmed the results previously obtained. Our data on the effects of anæsthesia on the



serum Ca of thyroid-fed and thyroidectomized animals were too few to permit of any conclusion. They were, however, much more variable than in normal an imals.

Iodine.—Ether anæsthesia for varying periods had no effect on the blood iodine of normal or thyroid-fed rabbits. Average values for 8 animals in each case w ere: Normal—9·4γ and 9·3γ per 100 c.c. before and during anæsthesia respectiv ely; thyroid-fed—63·9γ and 62·6γ respectively. A few experiments done on th yroidectomized animals suggested that in these also anæsthesia had no effect on the blood iodine. Other results from this Institute seem, however,

to indicate that anæsthetics, as administered in hospital practice to human patients, have some influence on the blood iodine (Anderson and Leitch, 1927).

Tolerance of the anæsthetic.—On the whole, the thyroid-fed and thyroidectomized animals took longer to become anæsthetized and bore the anæsthesia worse than normal animals. Artificial respiration was frequently necessary in the case of the former. When death occurred respiration always failed before the heart. Ability to bear the anæsthetic decreased with increasing doses of thyroid. It was noticed that those animals, thyroid-fed and thyroidectomized, that took ether badly, showed low pre-anæsthesia sugar values with little or no hyperglycæmic response and/or progressively increasing acidosis. Large doses of thyroid induced rapid onset of rigor mortis, which confirms some observations of Hoet and Marks (1926). The thyroid-fed animals were very excitable as a rule and more difficult to bleed, and the clotting time was noticeably diminished, but we were not able to make any accurate observations on this point.

DISCUSSION.

We have already observed (Magee and Glennie, 1927) that anoxæmia was not a factor in causing the changes in pH, sugar or P, so that they must have been due to the drug itself rather than to any interference with the respiratory function in its administration. This opinion in regard to the acidosis is in agreement with that of many earlier workers in anæsthetics.

The occurrence of an acidosis and hyperglycæmia in anæsthesia has been well known for a long time and has been substantiated by many recent observers. Data on the effect of anæsthesia on the blood P are, however, very scanty. Stehle and Bourne (1924), Martland and Robison (1924) and Potter (1925) have obtained results in accordance with ours.

In all the animals under ether there was a closer correspondence between the pH and the P than between the sugar and either of these. The precise reason for this relationship is not clear.

Very probably the lowering of the serum Ca played some part in the acidosis, since Leake *et al.* (1923) and Austin, Cullen *et al.* (1924) found a diminution of total base in the blood in ether anæsthesia. It is significant that we found only one paper where an increase in pH has been recorded and this was accompanied by an increase in serum Ca (Tomasson, 1926). It is also of interest that the anæsthesia was conducted on two physically sound but psychopathic women. Cloetta and Thomann (1924) and Pincussen and Dimitrijevic (1926) obtained results similar to ours. The return of the pH towards normal does not appear to have been previously observed. It is evidently a phenomenon of considerable practical importance, because its absence generally indicated some inability to bear the anæsthetic.

The fact that the blood iodine content was unaffected by ether would indicate that the secretory activity of the thyroid underwent no change, but it does not follow that the physiological action of the internal secretion in circulation remained unaltered during anæsthesia. The absence of hyperglycæmia in the thyroid-fed and anæsthetized animals indicates that the source of the excess sugar in normal anæsthetized animals was the liver glycogen. Further, the

lowered hyperglycæmic response to ether in the thyroidectomized animals suggests that the thyroid played some part in the mobilization of the excess sugar found in the blood of normal animals under ether.

SUMMARY.

The blood of rabbits was examined before and during ether anæsthesia in normal, thyroid-fed and thyroidectomized animals.

1. The inorganic phosphorus was lowest in March and April and highest in June and July.

2. Ether anæsthesia in normal animals lowered the calcium but increased the inorganic phosphorus, sugar and cH, the latter tending to return to normal after a time.

3. Increasing doses of thyroid first raised and then lowered the sugar. Thyroid feeding progressively, and thyroidectomy slightly, diminished the hyperglycæmic response to ether.

4. Large doses of thyroid increased the cH and inorganic phosphorus, which were still further raised by ether anæsthesia.

5. The effects of thyroidectomy alone and of thyroidectomy and ether on the cH and inorganic phosphorus were variable.

6. Thyroid feeding raised and thyroidectomy lowered the iodine. This was unaffected by ether anæsthesia in normal and thyroid-fed and probably also in thyroidectomized animals.

It was not possible for us to carry the above work any further towards a solution of the many points raised by it, but we considered that the data should be recorded.

We desire to thank Miss J. McCallum, B.Sc., for occasional technical assistance, and Mr. J. Morton, M.R.C.V.S., for some data on the seasonal variation of blood phosphate.

REFERENCES.

Anderson, Wm., and Leitch, I.—(1927) *Lancet*, **213** (II), 1391.
Austin, J. H., Cullen, G. E., Gram, H. C., and Robinson, H. W.—(1924) *J. Biol. Chem.*, **61**, 829.
Bodansky, A.—(1924) *Amer. J. Physiol.*, **69**, 498.
Burn, J. H., and Marks, H. P.—(1925) *J. Physiol.*, **60**, 131.
Cloetta, M., and Thomann, H.—(1924) *Arch. Exp. Path. u. Pharm.*, **103**, 260.
Hancher, K. G., Hupper, H., Blau, N. F., and Rogers, J.—(1925) *Amer. J. Physiol.*, **75**, 1.
Hess, A. F., and Lundagen, M. A.—(1922) *Proc. Soc. Exp. Biol. & Med.*, **19**, 380.
Hoet, J. P., and Marks, H. P.—(1926) *Proc. Roy. Soc.*, **100**, B, 72.
Kunde, M. M.—(1925) *Amer. J. Physiol.*, **76**, 225.
Leake, C. D., Leake, E. W., and Koehler, A. E.—(1923) *J. Biol. Chem.*, **56**, 319.
Leitch, I., and Henderson, J. M.—(1926) *Biochem. J.*, **20**, 1003.
Magee, H. E., and Glennie, A. E.—(1927) *J. Physiol.*, **64**, x.
Martin, C. J., and Lepper, E. H.—(1926) *Biochem. J.*, **20**, 37 and 1071.
Martland, M., and Robison, R.—(1924) *Ibid.*, **18**, 765.

Orr, J. B., and Leitch, I.—(1928) 'Iodine Metabolism.' H.M. Stationery Office (in press).
Pincussen, L., and Dimitrijevic, Il. N.—(1926) *Klin. Wchnschr.*, **5**, 849.
Potter, D. G. E.—(1925) *Quart. J. Med.*, **18**, 261.
Rabinowitch, I. M.—(1924) *J. Lab. & Clin. Med.*, **9**, 543.
Stehle, R. L., and Bourne, W.—(1924) *J. Biol. Chem.*, **60**, 17.
Tomasson, H.—(1926) *Biochem. Ztschr.*, **170**, 330.

THE PRINCIPLES OF CAPILLARY BLOOD-PRESSURE MEASUREMENTS AS APPLIED TO GLOMERULAR AND TUBULAR FUNCTION IN THE KIDNEY.

LEONARD HILL and JAMES M. McQUEEN.

From the National Institute for Medical Research, Hampstead, London.

Received for publication May 4th, 1928.

The first part of a previous paper (Hill and McQueen, 1921) dealt with a scheme of blood-pressure gradient in mammals between the aorta and the renal vein which was deduced from a consideration of data drawn from several sources. The main conclusions drawn from these were:

(1) The pressure gradient could be such that a normal flow of blood was maintained in a kidney, yet the effective capillary pressure in the glomeruli was insufficient to filter water and salts, etc., from the blood colloid.

(2) On obstructing the flow of urine in the ureter, the veins, capillaries and arterioles are compressed, and, when the maximum secretory pressure of the kidney is reached, these vessels shut up at diastole and open intermittently with each beat of the pulse, and blood flows then in them only so long as the level of systolic pressure overtops the compressing pressure. For this reason secretion ceases before the systolic pressure of the renal artery can be attained in the ureter. The high glomerular capillary pressure so induced was artificial and did not indicate the normal conditions. It was due to banking up—a phenomenon that has played an important part in all attempts to measure capillary pressure where a compression technique has been used.

The second part of the paper was due to L. Hill (1921), who, under the microscope, observed the glomerular capillary circulation in the frog's kidney,* and estimated the blood-pressure by observing that 20–35 mm. of Hg. pressure stopped the flow in the arterioles supplying the glomeruli, and 5–10 mm. Hg. pressure, quickly applied, sufficed momentarily to check the flow in the capillaries of a glomerulus. After injecting normal saline under the skin of the frog, he found the capsules to be so distended with a clear fluid as to be equal to, or even double, the volume of the glomeruli. The compressive force

* A. N. Richards, using a different method, observed the circulation in the glomeruli during the same winter, publishing his observations after the appearance of L. Hill's preliminary note.

then was transmitted to the glomerular capillaries through the fluid which distended the capsule. In one case, at a pressure a little higher than 5–6 mm. Hg., the blood entered the capillaries of the glomerulus in systole and was stopped entering in diastole, so that the flow became pulsatile. It was considered by Hill and McQueen that these measurements confirmed the existence of a low glomerular capillary pressure, and it was concluded that specific physiological action, not filtration or dialysis, controls the passage of fluid through the glomeruli.

Leonard Hill (1906) had pointed out that the intra-capsular fluid pressure normally must balance the glomerular capillary pressure, so that a very small excess of pressure maintains the flow of blood. The capillary pressure in the glomeruli effective for filtration must therefore be considerably lower than 5–10 mm. of Hg. L. Hill also pointed out that compression, except when momentarily applied, elicits the phenomenon of banking-up and raises the normal capillary and arteriole pressure to a higher level, viz. towards, or to, that of the artery which supplies the arterioles.

Nevertheless, H. L. White (1924), finding in three observations the osmotic pressure of the colloid of frog's blood to be from 7–9 mm. of Hg., and ignoring the balancing pressure of the fluid in the capsules, argued that the low capillary pressure values found by Hill were matched by equally low values of osmotic pressure, and that hence filtration could occur.

Later Krogh published direct osmometer measurements of frog's blood, and his figures range from 9 mm. of Hg. downwards, depending on the nature of the collodion filter used and the percentage of protein.

The protein content in frog's serum is given by the Japanese workers. Tamura and others (1927[3]), as ranging from 0·86 to 4·71%, and taking Krogh's 2·4 mm. of Hg. per 1% of protein, that brings the frog's osmotic pressure, as varying according to conditions, from 2 mm. of Hg. to 11 mm. of Hg.

With glomerular capillary pressures below 5–10 mm. of Hg. and osmotic measurements of frog's blood colloid ranging from 2 to 11 mm. of Hg. filtration is not proved, unless one were deliberately to choose within these ranges figures to suit one's purpose.

Landis (1928) has recorded observations on capillary pressure in the exposed mesentery of the frog, and these are so adjusted to White's figures that they support the theory of filtration. With 6 mm. Hg. of capillary pressure he noted inflow of fluid, with 10 mm. Hg. outflow from a capillary. While the accuracy of his method is open to doubt, he has left out of count the counter-balancing pressure which normally pertains.

Using a large hollow needle connected with a length of manometer tubing, the whole filled with Ringer solution save for an air-bubble index, L. Hill has found the pressure required to drive fluid into the abdominal cavity of a toad, sitting at rest, to be from 2–5 cm. H_2O. The pressure varied with respiration; struggling increased it. In an anæsthetized toad 1 cm. H_2O sufficed.

In the second part of this paper evidence is given that Landis's readings of capillary pressure are too high. Further observations accompanied by measurements of plasma osmotic pressures are required before Landis's conclusion can be accepted.

Not only White, but Cushny, in arguments in favour of filtration, have ignored what had all along been emphasized by L. Hill, both in kidney problems and in collateral problems connected with the brain, eyeball, salivary gland, etc., and by Hill and McQueen in publications on capillary pressure in man, and by McQueen in a paper on the production of tissue fluid, that it is essential to consider the influence of the countervailing pressure of the tissue fluid.

The fluid in the capsule of each Malpighian body has to traverse a long tortuous tube before reaching the pelvis of the kidney; from there onwards down the ureter to the bladder, the urine may be aided by the peristaltic contractions of the ureter. In the bladder the pressure is not atmospheric, but a positive one of varying degree. The abdomen of the living active animal is kept under a varying positive pressure by the tone of the abdominal muscles, the contractions of the abdominal viscera, and the action of the respiratory pump. The fluid in the capsules has then to overcome a resistance to onflow which may be, and probably is, no less than that which opposes the flow of blood from the glomerular capillaries to the veins. The whole kidney enclosed by a capsule of limited extensibility is throbbed by the systolic pulse, and the expansion of the kidney with each systole and its recoil with each diastole aids not only the exit of blood into the renal vein, but exit of urine from the tubules of the kidney into the pelvis. There pertains throughout the kidney a tissue fluid pressure counter to and almost balancing that within the capillaries and veins. The pressure in these vessels is greater by just that amount required to maintain the flow of blood, and, as shown by L. Hill, a very small pressure, *e.g.* 1–2 cm. H_2O, suffices to effect this. This balancing pressure, as elsewhere in the body, is regulated by the physiological imbibition of the renal cells and the secretory pressure produced by them within the capsule and tubules. In the Malpighian bodies there are certainly not the conditions which pertain in a filter or dialyser, viz. blood within capillaries under a relatively high pressure separated by a thin capsular membrane, with no pressure in the capsule on the other side of it. If the pressure of the fluid within the capsule almost counterbalances the pressure within the capillaries of the glomerulus, a physiological secretion must regulate the outflow of fluid and not an excess pressure within the capillaries. The Japanese workers, Tamura and others (1927[2]), have recently observed that when the pressure in the renal tubules and capsules rises as a result of ligation of the ureter, that within the glomerular capillaries rises *pari passu*, so that at an intra-ureteral pressure of 12 mm. Hg. the flow becomes pulsatile, being checked in diastole.

That the intracapsular pressure is significant is confirmed by the fact, noted by Richards and Schmidt (1922), that on pricking a distended capsule and letting the fluid escape into the interstitial kidney tissue, the glomerulus bulged markedly; obviously the intracapsular pressure counterbalanced the pressure of the blood, which was striving to distend the glomerulus.

Further, the Japanese workers have observed on provoking diuresis, the ureter being tied, that the capsules continued to distend when the flow of blood in the glomeruli had become pulsatile. Moreover, in frogs with ureter untied when diuresis ceased, the glomerular tuft retracted long before the distension of the capsule disappeared.

In the act of measuring stopping of flow pressure by compression with Roy and Graham Brown's apparatus one artificially raises capillary pressure. When one compresses a glomerulus of the kidney to stop capillary flow, that cannot be done till the flow in the afferent artery is stopped. So, too, the pressure within the tubules and capsules of the whole kidney is raised by obstruction of the ureter and secretion of urine; the pressure in the glomerular capillaries rises *pari passu* towards renal artery pressure.

When L. Hill and Flack (1912) injected saline into the aqueous chamber of the eyeball, the circulation in the eye continued, as shown by the outflow from a cortex vein until the arterial pressure measured in the carotid was just overtopped; the pressure in the capillaries rose *pari passu* with that of the fluid in the aqueous chamber. The same happens in the case of the brain enclosed in the skull.

The same principle applied, as shown by Hill and McQueen, when compression and blanching of an area of skin was used to determine capillary pressure in man.

It follows from this reasoning that compression equal to 5–10 mm. Hg., which was observed by L. Hill to render pulsatile and retard the glomerular capillary flow, was of necessity a measure of an artificially heightened capillary pressure. Now Hayman, in order to measure glomerular capillary pressure, first closed the tubule leading from a glomerulus by a glass rod, and then injected fluid into a capsule (after introduction of a quartz pipette) till he stopped the flow of blood. The pressure in the glomerular capillaries was thus artificially raised to the afferent artery systolic pressure, precisely as happens in the case of the capillaries of the eye on injecting saline into the eyeball.

Hayman then lowered the pressure until the flow returned in one capillary of the glomerulus and took this pressure to be the true capillary pressure. What he really measured was the pressure at which the crest of the systolic wave in the artery overcame the pressure in the capsule and produced a flow in the widest capillary—a banked-up pressure.

When L. Hill uses his index in measuring capillary blood-pressure he applies quickly a pressure which momentarily arrests the flow of corpuscles; if this compression is maintained the flow of blood is resumed by banking up of blood-pressure. By using this checking index instead of a stopping index, he secures accurate results. New observations have been made which confirm the previous ones. The results are given in the subsequent paper in this issue. Taking the check index as the true measurement of capillary pressure, and deducting the intracapsular pressure from the glomerular capillary pressure, it is clear that specific physiological action, not filtration, controls the passage of fluid through the glomeruli of the kidney. The check index is confirmed by other accurate methods of measuring capillary pressure.

Thus the method used by Basler (1912) was one of finding the counter fluid pressure required to stop bleeding from a small cut in the finger. This method gave 5–8 mm. Hg. In such a wound arterioles would be cut. Carrier and Rehberg (1923), in the laboratory of Krogh, found the pressure required to balance escape of blood from a capillary in the skin pierced by a hollow quartz needle observed by means of arc light and microscope, and obtained low

readings comparable to those obtained by the checking index of L. Hill. With the finger held about on the level of the clavicle the readings were 4½ cm. of water or 3½ mm. Hg. This pressure pertained in the large capillaries of the loops in the skin. Lower pressures were observed by L. Hill in the capillaries of the tadpole tail, in the 2–3-day-old chick embryo, and in the capillaries of adipose tissue in the mesentery of the mouse.

Krogh (1922) says—" It is impossible to doubt that the main resistance to be overcome lies in the arterioles, where, therefore, the main fall in pressure must take place, while comparatively insignificant pressure differences must suffice for the current through capillaries and veins." This is the view which one who has studied microscopically the capillary circulation is bound to take. It has been maintained for thirty years by L. Hill since his research on the cerebral circulation.

The Japanese observer Tamura visited England in 1921, and was shown the glomerular circulation by L. Hill. Since then he has carried out a series of researches, in which his co-workers have shared.

L. Hill observed in frogs not over-anæsthetized, and from which loss of blood was prevented by use of the cautery, that all the glomeruli in view showed an active circulation. In other cases some of the glomeruli showed little or no circulation. Tamura has observed in the resting frog's kidney active circulation in all glomeruli with no sign of distension of the glomeruli or capsules. He denies the presence of inactive glomeruli under normal conditions as described by Richards. The Japanese workers (1927[1]) find about 100 glomeruli, viewing the surface of the Japanese frog's kidney in the space between the adrenal body and the outer border from pole to pole. In the whole kidney there are 1000 or more. " Through all the glomeruli visible both in the kidney at rest and in the kidney during diuresis a circulation is carried on steadily," just as in the capillaries in other regions of the body the blood-flow in the glomerular capillaries is smooth and constant. An overdose of urethane or hæmorrhage produced non-activity of the circulation in some of the glomeruli as described by Richards. This the Japanese workers ascribe to the pressure in the capsules; a little fall in arterial pressure results in the capillary pressure dropping below the counterbalancing pressure of the capsular fluid.

In the kidney at rest, save for the Malpighian bodies near the outer border of the kidney, the glomerular surface was observed to be almost always in contact with Bowman's capsule. In such the secretion of urine may be reduced to 1 drop in 90′. As there is no sign of fluid being excreted in the capsule of the Malpighian body and reabsorbed in the tubules, the conclusion to be drawn is that there can exist an excellent circulation of blood in the glomeruli with no exudation of fluid into the capsules, as was deduced in Part I of our 1921 paper. If we try to explain these Japanese observations by the glomerular filtration theory, the colloidal osmotic pressure of the blood must be assumed to have been higher than the effective glomerular capillary pressure during rest, to become lower than this during diuresis. Our view is that during the rest of the kidney there was no diuretic substance in the blood to occasion a secretion of fluid. By secretion we mean a specific action of living cells as yet unexplained.

Richards found support of the filtration theory from his observation that in rest some of the glomeruli showed no sign of active circulation, while in diuresis the number of the active glomeruli increased until the blood flowed through all the glomeruli. But the Japanese observations, confirmed by L. Hill, now show that in the resting kidney with normal and unobstructed circulation all the glomeruli show an active circulation, while there is no sign of fluid in the capsule. Supporters of the filtration theory cannot well fall back on Cushny's "modern" theory that fluid is always passing into glomeruli where capillary pressure is high, and always being absorbed in the tubules.

The Japanese observers, as stated above, found that on tying the ureter of frogs and so obstructing the exit of urine, the glomerular capillary flow as the pressure in the ureter rises becomes pulsatile. The pulsatile flow becomes more and more marked with increasing intracapsular pressure till the glomerular circulation ceases in diastole and resumes in systole. By this observation they proved that the intracapsular pressure had overcome the diastolic pressure in the glomerular capillaries, and yet the Japanese found that occurred while fluid under influence of a diuretic was still being extruded into the intracapsular spaces and distending them. Having proved that fluid is extruded into the capsule during pulsatile glomerular capillary flow, they state "that the pressure difference between both sides of the glomerular capsule is of the same order of magnitude as that between the systolic and diastolic pressures in the glomerular capillaries, which is practically negligible." The observations of Landis (1925–26) show that the variation of pressure with systole and diastole is in the capillaries very small, *e.g.* 0·5–1·0 cm. H_2O.

The conditions in the Malpighian bodies with obstructed ureter are similar to those in the eyeball in glaucoma, where, owing to an inflammatory increase of fluid in the eyeball, the retinal arteries may be shut up in diastole to reopen in systole. The retinal veins can be seen to be pulsatile, hence the retinal capillaries are also pulsatile.

The Japanese observers noted that a pulsatile flow in the glomeruli also occurred on slightly bleeding a frog. In the same way in the eyeball in a patient when syncope is imminent, an arterial pulsation is visible in the retinal arteries due to the intra-ocular tension then being above the diastolic pressure in the retinal artery. The intra-ocular pressure in this case is above not only the diastolic pressure of the capillaries, but of the artery. The parallelism is striking and the problem is the same for eyeball as for kidney. But in the eye, the intra-ocular fluid is normally kept by secretion at a pressure of some 25 mm. Hg., in order to secure a tense and optically efficient eyeball. The capillary and venous pressure has just to overbalance the fluid pressure either in eye, kidney or other organ, to maintain a circulation.

Let us understand clearly what happens in the kidney when the ureter is tied. The intracapsular and tubular pressure rises, and the whole kidney becomes tense with fluid; compression of the renal veins follows, and owing to banking up, the pressure rises *pari passu* in the veins and capillaries. As the banking up proceeds, the glomerular capillary pressure rises towards the systolic pressure of its afferent artery, which in its turn rises towards that in the renal artery. In the early stages, when the increased intracapsular pressure just overtops the diastolic pressure in the glomerular capillaries, the

systolic pressure at each pulse comes into play and opens the capillaries and produces a flow for a short space of time. Whenever the capillary lumen is restored and the flow begins, the capillary pressure falls, and the intracapsular pressure closes them again. At the lower levels of intracapsular pressure at which pulsation first appears, and when the capsules are still becoming enlarged, the pulse pressure range is only very small—1 mm. Hg. or so. It must be borne in mind that the effect of an increase of intracapsular and intratubular pressure is gradually to approximate the diastolic level of pressure in the afferent arteries of the glomeruli towards the systolic. Hence the pulse-pressure range in these arteries becomes narrowed. As the intracapsular pressure overtops more and more the diastolic pressure, the glomerular capillaries are compressed for a longer period, and only the crest of the systolic wave is used to reopen the capillaries compressed in diastole. The diastolic stoppage of flow becomes of longer and longer duration.

Secretion ceases, as argued by Hill and McQueen and observed by Tamura, before the systolic pressure of the afferent arteries is reached in the ureter, through diminution of the blood-flow.

It must be borne in mind that the pulse pressure range is cut down markedly as the diameter of the lumen of the vessels carrying the blood diminishes. Thus nowhere in the normal capillaries is a velocity pulse observable. The Japanese noted that there was no evidence of a velocity pulse in the normal glomerular capillaries when secretion pertained. When the ureter was tied secretion continued, as shown by the increasing distension of the capsules at the time when the first signs of pulsation occurred in the glomerular capillaries—that is, when the pulse-pressure range was minimal, and there was no capillary pressure effective for filtration against osmotic pressures of blood colloid found to be as high as 2–11 mm. Hg. It may be noted here that the osmotic pressure of the plasma proteins of the mammal is calculated to be 25–30 mm. Hg., and the evidence shows that the capillary pressure is not higher in the mammal, but lower, than in the frog.

The Japanese workers note that the fluid so ingeniously drawn off for analysis by Richards and Wearn from the capsule is an unnatural fluid, as it is obtained by a cupping method. That may well be so when one notes that Wearn and Richards (1924) employed a suction of from 20–30 mm. of Hg. This suction pressure added to the glomerular capillary pressure might well produce a filtrate in the frog whose blood colloid osmotic pressure ranged from 2 mm. of Hg. to 11 mm. of Hg. That it would produce a pure filtrate is not likely, for Richards and Wearn allowed time for secretion into the capsule, which was going on at the time they pierced it with their capillary pipette. The quantitative analysis made of chlorides by Wearn and Richard showed in eleven cases chlorides in the glomerular fluid in excess of chlorides in the frog's plasma. Richards and Wearn justly conclude the filtration theory is not adequate to explain these results. It is noteworthy that similar salts to those obtained in the fluid from the capsule are to be found in the cerebro-spinal fluid and in the aqueous humour.

A theory which suggests itself, if dialysis comes into play at all, is that the convoluted tubules when stimulated by a diuretic secrete crystalloids which diffuse into the capsule, where they were found by Wearn and Richards,

and there draw water out of the glomerular capillaries by raising the osmotic pressure of the capsular fluid above that of the plasma. The membrana propria limiting the expansion of the glomerulus appears to be so arranged as to favour such an action. A second stage of the process might be absorption of crystalloids from the capsular fluid by the glomeruli. On blocking, or partially blocking the ureter, a continuity of fluid between tubules and capsules is established; the fall in concentration of chlorides, sulphates, urea, etc., which occurs in the urine secreted on the obstructed side, would then be due to a greater absorptive function of the glomeruli and tubules. On this theory, then, one of the strongest arguments used for proving absorption in the tubules solely would fall to the ground.

The observations of the Japanese workers given above have been extended by Miyamura (1927), who has found that reabsorption of water by the tubules is adjusted to the season and needs of the body. The secretion of urine no less than other secretions cannot then be explained by phenomena of filtration and dialysis studied on dead membranes. The membranes which separate the blood from the cells, no less than the blood and the cells, are ceaselessly varying in condition. Landis (1928) has observed that oxygen want, injury, and poisons such as urethane and alcohol, very greatly increase the permeation of fluid though the capillaries of the mesentery. C. S. Smith (1927) by observation and injection finds free anastomosis between the renal arteries and renal portal veins. The renal capillaries can be supplied by either system when one is wholly obstructed. Frogs and toads continue to secrete normal urine when kept alive after total obstruction of either system. Tamura (1927[3]) also has found secretion of urine re-established on the day following ligation of the renal arteries. As the pressure in the renal portal vein is under 10 mm. Hg., these observations confirm the view put forward in this paper. The increase of urinary secretion accompanying increase of blood-pressure which is observed in mammalian experiments may be justly ascribed to the increase of blood-flow and action of diuretics. The quantity and quality of the urine depends on the relation of the quality of the blood to the renal cells and the endothelium of the capillaries, and on the velocity of flow of blood through the kidney.

SUMMARY.

There is no evidence of a pressure in the glomerular capillaries adequate for overcoming the osmotic pressure of the plasma colloids and producing filtration of fluid into the Malpighian capsules. The fluid in the capsules counterbalances the pressure in the glomerular capillaries, and only a small excess of pressure in the capillaries suffices to maintain the flow of blood. There is evidence that while blood flows as usual through glomerular capillaries in the resting kidney, no fluid passes into the capsules. The distension of the capsules with fluid is brought about by specific action of diuretics.

REFERENCES.

Basler, A.—(1912) *Arch. f. d. ges. Physiol.*, **147**, 393.
Carrier, E. B., and Rehberg, P. B.—(1923) *Skand. Arch. Physiol.*, **44**, 20.
Hill, L.—(1906) *Biochem. J.*, **1**, 55.—(1908) 'Recent Advances in Physiology,' London (Arnold), p. 618.—(1921) *Brit. Med. J.*, **1**, 526.

HILL, L., AND FLACK, M.—(1912) *Proc. Roy. Soc.*, B, **85**, 439.
Idem., AND MCQUEEN, J.—(1921) *Brit. J. Exper. Pathol.*, **2**, 205.
KROGH.—(1922) 'The Anatomy and Physiology of Capillaries' (Yale University Press), p. 218.
LANDIS, E. M.—(1925–6) *Amer. J. Physiol.*, **75**, 548.—(1928) *Ibid.*, **83**, 528.
MIYAMURA, K.—(1927) *Jap. J. Med. Sci.*, IV. Pharmacology, **1**, 291.
RICHARDS, A. N., AND SCHMIDT, C. F.—(1922) *Amer. J. Physiol.*, **59**, 489.
SMITH, C. S.—(1927) *Ibid.*, **82**, 717.
TAMURA, K., AND OTHERS.—(1927[1]) *Jap. J. Med. Sci.*, IV. Pharmacology, **1**, 211.—(1927[2]) *Ibid.*, **1**, 249.—(1927[3]) *Ibid.*, **1**, 261.
WEARN, J. T., AND RICHARDS, A. N.—(1924) *Amer. J. Physiol.*, **71**, 209.
WHITE, H. L.—(1924) *Ibid.*, **68**, 523.

THE CAPILLARY BLOOD-PRESSURE IN RELATION TO ŒDEMA AND CEREBRAL AND INTRA-OCULAR PRESSURE.

LEONARD HILL.

From the National Institute for Medical Research, Hampstead, London.

Received for publication May 4th, 1928.

THE Roy and Graham Brown (1879–80) apparatus used for measuring the capillary blood-pressure consists of a small brass chamber floored with glass and roofed with a transparent distensile membrane. A convenient diameter is 3 mm. Into the chamber there is fixed a tube which is connected by a T-piece both with a water manometer and with a tube for producing a rise of pressure in the chamber and distending the membrane. The compression chamber is fixed to a flat piece of brass, which also carries a movable holder to which a piece of glass is attached of suitable size just to cover the membrane of the chamber when lowered into position. The glass can be fixed to the holder by sealing-wax and so adjusted in position. The holder is best raised and lowered by a screw arrangement so as to bring the glass nicely into position, just touching, but not compressing, the part under observation. E. J. Schuster kindly devised such an arrangement for me. A sheet of cork covers the brass floor of the apparatus, a hole being cut in it so that it fits snugly round the chamber. The cork is of such thickness as to bring the part of the frog under observation on a level with the membrane of the compression chamber.

The apparatus when in use is the same in principle as the sphygmomanometer which is employed for measuring blood-pressure in man, only the index of blood-pressure is obtained by microscopical observations of the vessels of the part which is compressed.

In using the Roy and Graham Brown apparatus it is necessary to take

precautions first that the blood-vessels are not to the least degree obstructed by the arrangement of the part under observation. Secondly, to use a membrane for covering the compression chamber, which while transparent is so distensile that very little pressure is required to make it sufficiently convex to compress the blood-vessels. The membrane most suitable for use is the pericardium of a rabbit put on loosely. It must not be stretched tight. This, after it has been tied on to the compression chamber, can be kept in good condition when not in use by covering with wool soaked in glycerine. The central tendon of the diaphragm of the rabbit is usable, but being more fibrous is less transparent. Suitable transparent membranes can be got also by stripping off the innermost (peritoneal) coat of the abdominal wall of the rabbit. A triple thick layer of the transparent part of the mesentery of the rabbit can also be used; this is perfect in distensibility and transparency, but is more easily broken than the other membranes.

The glass cover against which the blood-vessels are compressed by means of the membrane must be brought down so close that between it and the part under observation there is only a film of water or Ringer solution, but must not be brought close enough to exert of itself any compression upon the vessels. The glass cover when carefully lowered to the right position will not produce any visible blanching of, or slowing of the circulation in, an organ such as the tongue or kidney.

In observations on the tongue the frog is anæsthetized with urethane—0·2 c.c. of a 25% solution suffices injected under the skin of the back. It is fixed belly downwards on a sheet of cork with the mouth pointing to the chamber of the apparatus. The mouth is opened and the tip of the tongue drawn out, and a thread tied to either side of this. By means of these threads and pins the front part of the tongue is then spread out over the membrane of the apparatus. The level of the cork board is arranged so that the tongue is on the same level as this membrane. The mouth is held open by a pin, so that the blood-vessels of the tongue are quite free from any obstruction. In dealing with the web, threads are tied on two of the toes, and these are drawn apart and tied to pins so as to bring the border of the web over the membrane of the apparatus. The glass cover must be of suitable shape so as not to touch the toes while covering the observed portion of web. In the case of the kidney, the frog is anæsthetized with as small a dose of urethane as suffices (*e. g.* 0·2 c.c. of a 0·25% solution), and then fixed on its side with legs extended. The skin is cut over the pelvis of the upper side of the animal by a crucial incision and bleeding prevented by use of the cautery. The pelvis on that side is then removed by means of strong scissors, cutting through the bone first below and then above; all bleeding is stopped by the cautery. The kidney is thus exposed, and the renal-portal vein is seen running along its outer border. The membrane attached to this border in the middle part of the kidney is then picked up in the forceps—it does not matter if the vein is included—and a ligature tied on to it. A hook (bent pin) with thread attached is also fastened to the abdominal wall. The frog is then put upon its back and fixed on the cork board of the apparatus so that the kidney is on a level with the membrane of the compression chamber and close to its edge. By gentle traction on the attached thread the outer edge of the kidney is then

brought over the membrane. The renal arteries which enter the inner edge allow enough play for this to be done without obstruction of the circulation. The abdominal wall is now drawn out of the way by means of the hook and thread, and the glass cover then lowered till just in apposition with the surface of the kidney, which is wetted with Ringer solution. Glomeruli can now be observed in active circulation on illuminating the kidney with the arc light and using an inch objective. A slight degree of compression may be required to express the streams of renal-portal blood and bring the glomeruli and their afferent arterioles clearly into view. For success it is imperative that the animal should not be over deeply anæsthetized, that little or no blood should be lost and that no excessive traction should be put on the kidney. The kidney should appear crimson with blood. Then, as Tamura (1927) says, all the glomeruli in view will be found in active circulation. If water be injected under the skin some hour or two beforehand the capsules may appear distended with fluid and much larger than the glomeruli.

A number of measurements made by the Roy and Graham Brown method on the web, tongue, mesentery and kidney of the frog when in the best condition show that the compression required to stop the terminal arteriole and its capillaries in systole is approximately 20 mm. Hg. Further the pressure which suffices to momentarily arrest the flow of corpuscles in the capillaries with fastest flow and in the terminal arteriole supplying these is about 10 mm. Hg.; the same pressure (or less) if maintained will be found to retard or almost stop the flow in some or other capillary in the field of view where the resistance is highest, while having no obvious effect on the wider capillaries wherein the flow is fast. About the same pressure, viz. 10 mm. Hg., when maintained suffices to pale slightly the arteriole feeding the capillaries and just produce evidence of a velocity pulse. To produce this effect, normal pressure within the arteriole must be just surpassed. A very slight and negligible degree of banking up occurs in its production, as in the case when the momentary check index is used. A marked degree of pulsatile flow is produced by a higher degree of compression, for banking up then occurs owing to the obstructed blood-flow and conversion of kinetic into static energy. If a main arteriole supplying terminal arterioles be in the field of view, it will be found that a momentary check may be brought about by about 15 mm. Hg. of compression quickly applied, and that a velocity pulse may just become evident when the same degree of compression is maintained. A pressure of 25–30 mm. Hg. may be required to stop the flow in systole of this main arteriole. Tamura found this pressure in the renal artery of the frog by direct measurement.

E. M. Landis (1925–26), referring to the compression method, suggests that momentary pressure producing a partial occlusion of all the veins in the area might slightly modify flow in a few capillaries without equalling even venous pressure. He also suggests that the higher capillary pressures found by L. Hill are in organs where masses of tissue like kidney or tongue have to be compressed.

The index of modification of flow used by L. Hill is the momentary complete stoppage of the corpuscles, and as this pressure when maintained gives the velocity pulse or flicker index, and may also suffice to arrest the flow in

one or more capillaries in the network, there is no reason to doubt its accuracy.

Landis has made measurements of the pressure in one or other of the blood-vessels of the frog's mesentery, introducing a quartz pipette within the lumen for this purpose, and finding (1) the pressure required to cause a dye to flow into the vessel all the time except at the height of systole when two or three corpuscles would be pushed into the tip of the pipette—this was taken as the index of the systolic pressure; (2) the pressure at which there was a periodic arrest or marked slowing of flow of corpuscles into the pipette—this was taken as the index of diastolic pressure. The mean pressure was taken as the half value, or that pressure at which the corpuscles fluctuated to and fro.

There are possible errors in this method. Owing to banking up due to forcing in of fluid the systolic pressure must be that of the larger vessel supplying the one under observation.

Landis states that the "orifice" of the pipette measured 4–8μ, and that it was introduced so as to be as near as possible to the endothelial wall, and obstruct the flow to the least degree. The question arises as to whether "orifice" includes the whole diameter of the pipette. The diameter of the capillaries is given by Landis as 10–30μ. It is evident, then, that the pipette may have occupied a third or more of the lumen, and by partly obstructing the flow raised the pressure above normal. Landis himself gives figures which show how any obstruction to flow notably causes banking up and rise of pressure. Thus when a capillary was stopped the pressure on the arterial side was found by his method to be 15 cm. H_2O and on the venous side 10·2 cm. H_2O, while very high values of 22–29 cm. were found when venous outflow was stopped. The obstruction of a single fairly large venule resulted in a local rise of 3–4 cm. H_2O.

Only a few capillaries are to be found in the mesentery of the small intestine of the frog, arranged in a branching network. These are close to the gut. There are also straight vessels generally unbranched running parallel to the arteries wider than ordinary capillaries and with very fast flow. These have a wall resembling an ordinary capillary, but are like terminal arterioles both in size and velocity of flow. It seems probable that these vessels are those which Landis calls arterial capillaries, in which the readings were higher than in what he calls venous capillaries.

Landis used a more accurate method of measuring the pressure in the case of branching capillaries. By introducing a pipette into one branch he measured the pressure in the capillary from which the branch arose, this taking on the part of a side tube. In this case the pipette would not obstruct the flow, and the systolic pressure would be that of the capillary from which the branch arose. Landis unfortunately does not separately tabulate the figures given by the two methods, but states that over half his readings of capillary pressure lay between 10 and 15 cm. of water with 13 H_2O as the most frequent. The lowest capillary pressure was 5, the highest 22 cm. H_2O. If it is assumed, as appears just, that the highest measurements were taken in the unbranched vessels with fastest flow by introduction of the pipette into the lumen, then a deduction must be made for a partial obstruction of flow by the pipette. As according to the evidence of L. Hill half the systolic pressure appears to be

spent in maintaining velocity of flow, the figures obtained by Landis may be lowered justly and may approximate to those found by L. Hill—viz. about 10 cm. H_2O.

In the mesentery attached to the stomach and duodenum of the toad there may be seen networks of capillaries supplying transparent parts of the pancreas. These supplied by arterioles unite to form portal venules; the whole makes an admirable subject for study.

The systolic pressure in the arterioles is found, as elsewhere, to be about 20 cm. H_2O by the compression method, while the "check" and velocity pulse index give a pressure of about 10 cm. H_2O, and this pressure suffices to stop or almost stop some capillary or capillaries in the network where the flow is naturally slower.

Average pressures taken from a very large number of observations on terminal arterioles and arterial capillaries are given in the table:

	Pressures in mm. Hg.	
	Stop in systole.	Flow momentarily stopped and velocity pulse made visible.
Frog's web	23	8
„ tongue	23	9
„ kidney, glomeruli	24	8
„ mesentery	21	8
„ bladder	23	7
Toad's lung	21	10
„ liver	2 (portal venule reversed)	...
„ pancreas	22	9
Tadpole's tail	9	4
Mouse's mesentery	23	9
„ abdominal wall	25	9
„ ear	20	8
Bat's wing	18	7
2–3-day-old chick embryo	2	...

When the whole of a part, say the end portion of a lung, or tongue of the frog, is under compression there is no alternative path open, and the same pressure, therefore, is required to stop the flow in the artery, arterioles and capillaries.

There is no noteworthy difference between the measurements of, say, the fast-flowing spiral capillaries of the tongue of the frog and those of the glomeruli of the kidney. A lower pressure suffices to retard or stop the flow in outlying capillaries where the resistance is greater. Lower pressures are found in the case of the liver and the tadpole's tail. In these, the flow in capillaries may be stopped by compression of 2–3 mm. Hg. Observations have been made on the warm-blooded animal, using the bat wing, the ear,

mesentery and abdominal (muscular) wall of the mouse and the 2–3-day-old chick embryo. In the case of the 2–3-day-old chick embryo, the egg was opened in a bath of warm Ringer's solution so as to expose the embryo and vascular area, and a cut with scissors made through the membrane all round this. By means of a large section-lifter, the embryo and its vascular area were detached and raised, and floated on to a sheet of cellophane (acetyl cellulose), which surrounded the membrane of the compression chamber, so that part of the vascular area lay upon the membrane. The cellophane rested upon a brass ring-shaped box which surrounded the chamber of the compression apparatus, and was kept at 39° C. by a water circulation. Warm Ringer was used to irrigate gently the edge of the embryonic area. It was found that a compression of 2 mm. Hg. sufficed to stop the arterioles in systole, and that this small pressure was sufficient to maintain the circulation in the embryo. The functions of the living organism proceed then when the capillary pressure is very low.

To observe the mesentery, a small mouse is anæsthetized with urethane and placed on a shallow brass trough which is kept warm by an electric coil; the abdomen is opened so as to allow a small loop of intestine to be drawn out freely; the mesentery is arranged to lie over the membrane of the compression apparatus, while the gut rests on a pad of wool kept wet with warm Ringer's solution. The capillaries at the edge of a minute lobule of fat can then be observed under the high power, a small arc light being used as a source of illumination. It was found that about 2–3 mm. Hg. sufficed to stop the flow in some of these capillaries when 23 mm. Hg. stopped the flow of the arteriole in systole. To observe the circulation in the abdominal wall, a small white mouse is anæsthetized with urethane; after reflecting one-half of the skin of the abdominal wall by an incision carried first up the middle line and then across one side of the abdomen just below the thorax; the abdominal muscular wall can be reflected by a similar incision, and so brought on to the membrane of the compression chamber without material damage to the circulation in the blood-vessels which enter the flap from below. The cautery is used to stop loss of blood.

In three mice the main artery of the flap was stopped in systole by 47, 52, 40 mm. Hg., one of the main arterioles by 26, 26, 24 mm. Hg., a terminal arteriole by 18, 18, 18. In this preparation the blood was able to take the path through other vessels not under compression, and a reversal of flow might be produced, the blood being expressed up an arteriole into a main arteriole wherein the blood was flowing to the uncompressed part. Under the high power, the blood was seen to be flowing with such speed through most of the inter-muscular capillaries that the corpuscles were not visible. In some loops, where the flow was slow enough to be observed, it was retarded or stopped by a pressure of 4–7 mm. Hg. In the fast-flowing capillaries the same pressure was required to stop the flow in systole or to momentarily stop it as in the terminal arteriole. This is found to be the case in all parts of the body examined.

Hibernating bats were obtained, warmed up into activity and anæsthetized with urethane, and the vessels of a part of the membrane of the wing arranged for observation and measurement. The diameter of the vessels which were

compressed was measured in the bat's wing by means of a micrometer eyepiece and found to be—

Main arteriole	about	0·08 mm.
Terminal „	„	0·017 „
Main venule	„	0·16 „
Terminal „	„	0·034 „

The pressure required to stop the main arteriole in systole was from 35–50 mm. Hg.

The terminal arterioles arise from the main one almost at a right angle. These were stopped in systole by 12–20 mm. Hg. There was then a notable drop in pressure from the main to the terminal artery accompanying a diminution in diameter from 0·08–0·017 mm. The capillaries were observed under the high power, and a pressure of 2–6 mm. Hg. sufficed to greatly retard or stop the flow in those where the resistance was greatest and the natural rate of flow least.

When the pressure is measured in man in the brachial artery by means of the sphygmomanometer banking up is produced, just as it is in the vessels of the web, tongue, etc., by use of the Roy and Graham Brown apparatus. The systolic pressure measured by stoppage of the pulse is therefore that in the subclavian artery. The true lateral pressure in the brachial artery is given by the pressure which just produces a velocity pulse; that will be approximately the pressure at which the sudden change of sound occurs when the diastolic pressure is measured by auditory means, the stethoscope being applied to the brachial artery. This pressure indicates the diastolic pressure, not in the brachial, but in the subclavian artery. It indicates the mean pressure in the brachial artery when the flow of blood is unobstructed.

The Roy and Graham Brown apparatus allows the visual observation of all the phenomena which occur when the method of measuring blood-pressure in man is used, and is, therefore, instructive to the student. It should be a class instrument; the circulation in the web, tongue, lung, mesentery, pancreas and liver of the frog can easily be studied by its means.

The effect of banking up can be shown by first measuring the pressure which stops an arteriole in the web, *e. g.* 17 mm. Hg. A ligature is then drawn round the thigh so as to compress it sufficiently to make the flow in the web a pulsatile one. The pressure now required to stop the arteriole in the web is raised, *e. g.* to 24 mm. Hg. This is because all alternative pathways of the flow are blocked by the ligature, and thus all the vessels are banked up to the pressure in the main artery in the thigh.

The effect on blood-pressure of pithing the medulla and spinal cord of a frog can be shown by means of the apparatus. Thus the pressure in the main arteriole of the web of a frog fell from 25 to 11 mm. Hg., while the vessels were seen to be much paler. Later the pressure rose to 14.

When the arteries in the thigh of a frog are tied so that the circulation is stopped in the web, it will be found that a compressure of 1 or 2 mm. Hg. suffices to send the corpuscles flowing along the capillaries and veins at a rate resembling that of the natural flow. This fact shows how slight is the resistance in the capillaries to the normal rate of flow. The fact that the lateral pressure

in the arteriole as indicated by the momentary check index is about half that of the pressure required to stop the flow in systole shows that about half the pressure in the small artery from which the arteriole arises is spent in maintaining velocity of flow.

A comparison of the conditions of the capillary circulation in different organs can now be given.

In the eye the pressure of the intra-ocular fluid is raised to some 25 mm. Hg. in order to keep the eye-ball tense, confined as it is outside by the rigid sclerotic coat, and inside by an internal membrane. The intra-ocular pressure maintains the shape of the eye, as is fitting for an optical instrument. L. Hill has maintained that the cells lining the ciliary processes regulate the secretion of aqueous which maintains this special function. Duke-Elder recently has supported the theory of Leber that the aqueous is a filtrate produced by pressure in the capillaries sufficient to overcome the osmotic pressure of the plasma colloids, and this makes its production a function of the heart. In all parts of the body, however, whether of a lowly organism without circulation, or of an animal with circulation, we find special secretions carried out by special cells. Watery secretions are produced by organisms in which there is no circulation and the question of filtration or dialysis cannot arise. Duke-Elder (1926), by most skilful means, has measured the venous and arterial pressures in the retinal vessels. The retinal venous pressure is, he finds, just a very little higher (1–2 mm. Hg.) than the intra-ocular pressure, for blood can be seen issuing from a retinal vein when this is pricked by a needle introduced within the eye-ball; in some cases the blood only escapes in little gushes synchronous with each systole. "The venous pressure varies," he says, "directly and very intimately with the intra-ocular pressure." He agrees in this respect with the views of L. Hill and M. Flack (1912[2]). Just as the venous sinuses in the skull are protected by extensile dura mater, so the canal of Schlemm in the eye-ball is shielded by the inextensile sclerotic coat within which it lies; the pressure therein may fall therefore below that of the intra-ocular pressure when a rise of this pressure occurs. The canal of Schlemm, no less than the venous sinuses in the skull, is influenced by changes of pressure in the veins outside the eye-ball. To measure the retinal arterial pressure Duke-Elder (1926–7) introduced into the eye a fine quartz tube, guided it into a retinal artery, and then measured the pressure required to drive a coloured fluid into this artery. He thus found, as he thought, the mean gradient from retinal artery to retinal vein to be more than 50 mm. Hg. "No method," he says, "has yet been devised to measure the capillary pressure in the eye by any intra-ocular manipulation," and then says, in agreement with L. Hill, "a pressure applied to the eye at once affects the venous pressure and with it the capillary pressure, the three tending to rise coincidently." "Most recent estimations," continues Duke-Elder, "show that the capillary pressure is very low, but the majority of these have been undertaken in the skin, most of whose capillaries appear to be venous in nature." "There seems little justification," he says, "in applying them to the general circulation, and none for applying them to the specialized conditions of the eye." He then goes on to point out that "the ciliary arteries break up into at once a rich network of capillaries. The veins are physiologically constricted at their

exits from the eye, and the whole system is confined by the sclerotic with an intra-ocular pressure of 20–25 mm. Hg.," and concludes with the statement that "these conditions make it probable that the pressure in the arterial capillaries rises at least 30 mm. Hg. higher than that in the chamber of the eye, *i. e.* to a total of about 50–55 mm. Hg. This difference is required to effect the almost protein-free dialysate which the aqueous humour is." While this claim for so high a capillary pressure in the eye is unsupported by evidence, the many measurements made in other parts of the body go far to refute it.

When Duke-Elder forced a coloured fluid to enter a retinal artery, he stopped the flow of blood in the artery and measured the systolic pressure banked up by arrest of blood-flow. The true pressure in the retinal artery is much lower and nearer to the intra-ocular pressure. The check index would probably show it to be about half the figure given by Duke-Elder.

The conditions in the eye-ball may be compared to those in the kidney when the ureter is obstructed. There are the rich network of capillaries in the glomeruli, the fluid in the renal tubules and Malpighian capsules at a pressure of 12 mm. Hg., and the capsule of the kidney confining the whole becoming inextensile with a certain degree of swelling of the whole organ. In the glomerular capillaries there is no evidence of a pressure 30 mm. Hg. above that in the capsules; quite the contrary. Tamura observed that when the capsular pressure reached 12 mm. Hg. by telling against that in the capillaries it produced a pulsatile flow. The evidence brought forward by L. Hill shows that the normal pressure is indicated by the first sign of a pulsatile flow, and that the capillary pressure in the glomeruli is, under normal conditions, less than 12 mm. Hg.

It was shown by L. Hill and M. Flack in the case of the eye, that if the aqueous humour be let out and then the belly be compressed so as to congest the veins and capillaries of the iris, the iris bulges forward and hæmorrhages break out on its surface. This does not occur in the normal eye, because the pressure of the aqueous confined by the rigid coat of the eye rises *pari passu* when the veins congested by the pressure on the belly begin to swell, and this, counterbalancing the increase of pressure in the vessels of the iris, limits swelling of this structure and so prevents the bursting of the vessels and hæmorrhages. In the kidney the pressure in vessels of the glomeruli automatically balances any rise of intra-capsular pressure produced by secretion, and the pulsatile expansion of the kidney with each systole of the heart not only keeps going the circulation, but helps to impel the fluid in the capsules down the tubules. So, too, the pulse in the eye and in the brain aids the circulation both of blood and tissue fluid, whether it be aqueous or cerebro-spinal.

In the case of the brain, naturally enclosed in a rigid skull, L. Hill (1896) pointed out that when the skull is trephined and the dura mater incised, the brain bulges into the hole because the pressure in the vessels of the brain is no longer balanced there as it is normally by a film of cerebro-spinal fluid contained within the intact skull. By screwing in his brain-pressure gauge and restoring by means of it the normal position of the brain as it lies in the closed skull, the pressure in the small blood-vessels which cause the bulging of the brain was measured. It was found to be about 5–10 mm. Hg. in the animal resting

horizontally. The pressure of the cerebro-spinal fluid was found to be approximately equal to that of the cerebral veins where they enter the sinuses within the dura mater. Measuring it at the level of the occipito-atlantoid membrane, Weed and McKibben (1919) found it to be a little higher, *e. g.* 30 mm. H_2O (some 2 mm. Hg.) higher than the pressure in the sagittal sinus. The effect of gravity on the fluid contributes to this difference, as does the suction action of respiration acting on the veins outside the skull into which the sinuses open. Weed states that the pressure of the cerebro-spinal fluid suffices to stop hæmorrhage from a small cut made in the sagittal venous sinus after the skull has been closed, and the animal kept alive for a day or two. The pressure in the capillaries and veins in the pia mater must be a little greater than that of the cerebro-spinal fluid—just enough to keep the circulation going, no more. There is no justification for the assumption of a pressure of 30–40 mm. Hg. in the cerebral capillaries.

Whatever the secretory pressure of the cerebro-spinal fluid may be, the capillary pressure and venous pressure must rise to counterbalance it and just over-balance it—as in the case of the eye—otherwise the capillaries would be compressed and the circulation stop. When Weed and Hughson (1921) injected a hypotonic salt solution into the blood, the brain tissue swelled through imbibition. Injection of hypertonic solution had the opposite effect, shrank the brain and allowed more blood within the skull. The same happens in the case of the eye-ball (Freemont-Smith, 1927). Weed found that the pressure of the cerebro-spinal fluid was higher than that in the sagittal sinus normally, and also after a hypotonic injection; the reverse, he says, was the case after a hypertonic injection. In the former case there is uptake of fluid by the brain, the cerebral veins are compressed, and the capillary venous pressure rises to balance the greater pressure of the fluid and the blood-flow becomes faster. In the latter case the brain shrinks, giving up fluid, and the capillary venous pressure falls, and more blood collects within the cerebral blood-vessels and the flow is more sluggish. When a part of the brain is compressed, *e. g.* by a clot from a ruptured meningeal artery, the pressure rises in the compressed vessels to systolic pressure, and the cerebral pressure is thus increased in and around the compressed area.

Of peculiar interest is the case of the submaxillary salivary gland. L. Hill and Flack (1912[1]) showed that there is a structural contrivance which allows the pressure in the ducts to rise higher than that in the carotid artery without occlusion of the veins within the gland. The whole gland is confined as other organs are within a capsule which, after allowing the gland a limited degree of expansion, becomes inextensile. The cells of the ducts and alveoli are also confined by a membrana propria which limits their expanding beyond a certain amount.

When the salivary duct is connected with a mercury manometer and the chorda tympani stimulated, the pressure of the saliva, as is well known, may rise considerably higher than the carotid pressure. The gland then feels very tense. Under these conditions blood will be found still flowing from the efferent veins of the gland. As the alveoli swell through obstruction of secretion the veins and capillaries are compressed, and the pressure rises *pari passu* in these up towards arterial pressure with narrowed lumina of veins and

accelerated flow. Finally, the membrana propria become taut, and as the vessels are now shielded from further excess pressure in the alveoli, the circulation continues. This special arrangement has been evolved probably in order to allow secretory pressure to overcome obstruction to outflow in the mouth.

The capillary circulation can be observed in the edge of a lobule of the liver of a small toad, as pointed out by McQueen (1927). The blood there moves slowly, and in the hepatic venules may be checked by each systole of the auricle which dams back the blood in the hepatic veins. In many capillaries the blood appears to be in temporary stasis. This is so, however carefully the preparation is handled. A compression of 2–3 mm. Hg. stops the inflow, while it sends the blood running up the veins, to return into the capillaries when compression is removed. Struggling has the same effect. The experiment shows how important is respiration and the compressive action of the abdominal wall and diaphragm for keeping up a circulation in the liver.

In the case of the body-wall or a limb, the pressure in the capillaries and veins is counterbalanced by the imbibition pressure of the tissues, these being confined by aponeuroses, and by the skin. The veins, provided with valves, are so arranged that every movement squeezes blood from the capillaries and venules up the veins towards the heart. Gravity due to changes of posture aids the pumping action of the muscles. Thus the hydrostatic pressure due to posture does not appear in the capillaries, *e. g.* of the foot, except when the leg is hung down and kept motionless. The discomfort produced by swelling of the veins, etc., occasions movement and naturally prevents hypostatic congestion. When the veins of the upper arm are obstructed by the cuff of a sphygmomanometer in which a pressure is maintained a little below that of the systolic pressure, the pressure in the big veins quickly rises to that level—that is, a little below the systolic pressure of the subclavian artery due to banking up. This pressure quickly pertains in the widest connecting channels between arteries and veins, but not in the whole capillary field of the arm. It takes long to fill and distend the whole field, and discomfort becomes so great that the experiment must be ended before this happens. It cannot justly be assumed, then, that the pressure in all the capillaries rises to that in the big veins in such an experiment. Blood can be pushed out of a small capillary area by a lower pressure than this because the whole field is not full.

When the imbibition pressure of tissues confined by connective tissue aponeurosis or bone is increased and the arterioles are dilated by inflammation, there results increased pressure in the part (turgor) and throbbing is felt with each pulse. While the veins of the part are compressed the arteries are dilated. In the first stage of inflammation the increasing tissue pressure due to imbibition is counterbalanced by the rising pressure in the capillaries and veins and the blood-flow is rapid. This reaction is benefited by hot fomentations, which soften and relax the confining skin and connective tissues and allow more blood and tissue lymph to come into the inflamed part; when inflammation proceeds to stasis and formation of pus, the condition is relieved by cutting and letting out the killed tissue products. This relieves tension and compression of surrounding blood-vessels and allows lymph to exude from these.

In every part of the body, then, the counterbalancing pressure of the tissues comes into play. On this depends the firmness and tone of the body, the good condition which is felt on pinching up or pressing in the skin, and the maintenance of an effective circulation. In the condition of shock there is loss of this tone and the circulation tends to fail, the blood sinking into flaccid dependent parts. Starling (1909) stated that "the average capillary pressure (in mammals) may be taken to be as something between 20 and 30 mm. Hg." Such pressure is required to overcome the osmotic pressure of the plasma colloids. There is no evidence of such pressure, and therefore theories concerning the filtration of tissue fluid from the capillaries fall to the ground. So, too, in the case of pathological theories concerning "back-pressure" and œdema. Stagnation of flow, want of oxygen and change of tissue imbibition and endothelial membrane brought about thereby are causes of œdema, not "back-pressure."

Landis found that dyes penetrate the capillary wall at low pressures after injury by want of oxygen, urethane, ether or alcohol. The penetration is not due to increase of porosity by dilatation as Krogh surmised, for it occurs without any sign of dilatation.

All the known facts fall harmoniously into line with the view that the permeability of the endothelial wall, varying with state of the tissue-cells and the blood, controls the passage of fluid between the blood and the tissues, and that the actual height of the capillary pressure is relatively of little importance.

SUMMARY.

The capillary pressure is measured by means of the Roy and Graham-Brown apparatus, using the momentary stoppage of the flow as the index, or the first sign of a velocity pulse. Stoppage of flow in systole produces banking up of the pressure to that in the artery supplying the compressed part. More than half the pressure in this artery is spent in maintaining velocity of flow in the arterioles and capillaries. The pressure in the capillaries is counterbalanced by that of the tissue fluids, and the excess of capillary pressure is insufficient to overcome the osmotic pressure of the plasma colloids and produce filtration of fluid. The passage of fluid in and out of the capillaries is controlled by changes in the endothelial membranes, blood and tissue-cells.

REFERENCES.

Bayliss, W. M., and Hill, L.—(1911) "The Experimental Pathology of the Cerebral Circulation," in *Allbutt and Rolleston's System of Medicine*, **8**, 30.
Duke-Elder, W. S.—(1926) *J. Physiol.*, **61**, 409.—(1926–7) *Ibid.*, **62**, 1, 315.
Freemont-Smith, F.—(1927) *Arch. Neurol. and Psych.*, **18**, 550.
Hill, L.—(1896) 'The Physiology and Pathology of the Cerebral Circulation,' London.
Idem., and Flack, M.—(1912[1]) *Proc. Roy. Soc.* B., **85**, 312.—(1912[2]) *Ibid.*, **85**, 439.
Landis, E. M.—(1925–6) *Amer. J. Physiol.*, **75**, 548.
McQueen, J.—(1927) *Brit. Med. J.*, **2**, 1137.
Roy, C. S., and Brown, J. G.—(1879–80) *J. Physiol.*, **2**, 323.
Starling, E. H.—(1909) 'The Fluids of the Body.' London (Constable).
Tamura, K., and Others.—(1927) *Jap. J. Med. Sci.*, IV. Pharmacology, I, 249.
Weed, L. H., and Hughson, W.—(1921) *Am. J. Physiol.*, **58**, 101.
Weed, L. H., and McKibben, P. S.—(1919) *Ibid.*, **48**, 331, 512.

THE EFFECT OF RADIATION ON ROUS CHICKEN TUMOUR.

J. C. MOTTRAM.

From the Research Laboratory, The Radium Institute, London.

Received for publication May 9th, 1928.

In a previous paper (1926) it was shown that although Rous chicken tumour is very resistant to β radiation applied *in vitro*, tumours radiated *in vivo* are destroyed by relatively small doses not much greater than is required to destroy mouse and rat tumours. Further researches have been carried out to account for this remarkable difference between *in-vitro* and *in-vivo* exposures.

The first experiments were designed to see whether tumours radiated *in vivo* had lost their power to infect by a cell-free filtrate. A chicken was scarified in four places and tumour emulsion applied. In 14 days the following tumours resulted: 8×8, 3×3, 17×10 and 13×11 mm. The two smallest tumours were radiated for $1\frac{1}{2}$ hours to a radium application (110 mgm. $RaBr_2 4H_2O$, area 2×2 cm., screen 0·12 mm. silver). One hour's radiation to this applicator causes such tumours to disappear. Three days later the tumours were removed, minced and dried over phosphoric acid at reduced pressure. A fresh chicken was then scarified in four places, and the four dried and ground tumours applied; 10 days later all the areas were positive.

This was repeated, 2 hours' radiation being given, and the tumours removed 4 days later with a positive result, and again with 3 hours' radiation 5 days later. In the next experiment with 2 hours' radiation, 3 days later a clarified cell-free emulsion was used, the tumour emulsion being diluted to 1 in 20, and lastly this was repeated carrying the dilution to 1 in 160. All these were positive.

It is evident that the disappearance of tumours radiated *in vivo* is not due to the destruction of their power to infect by cell-free filtrates.

In a second series of experiments, areas of normal skin were radiated, scarified and tumour filtrate applied. An area of skin on the right side was radiated for 2 hours; immediately afterwards this area and a control area on the opposite side was scarified and tumour filtrate applied; 17 days afterwards there was a tumour on the control area of 13×12 mm. and none on the radiated area. A second experiment in which the radiation was for 1 hour

gave a similar result. In a third experiment 4 areas were radiated, two for 1 hour and two for ½ hour: the following table gives the results:

	6th day.	10th day.	14th day.
Control . . .	0	12 × 11	17 × 14
,, . . .	7 × 7	18 × 14	26 × 16
Radiation 1 hour .	...	...	0
,, ,, .	...	12 × 10	12 × 12
,, ½ hour .	...	14 × 7	17 × 12
,, ,, .	...	14 × 12	19 × 12

The figures represent millimetres. 0 = small nodule.

It is evident that 2 hours' radiation to the skin makes it unable to support the growth of Rous tumour, 1 hour is on the border line, whilst ½ hour's radiation is insufficient. Here, therefore, is an explanation for the disappearance of tumours radiated *in vivo*: the tumour-cells are killed by the radiation, and though the power to infect by cell-free filtrate is not destroyed, the surrounding normal tissues are rendered non-receptive.

A series of histological examinations of scarified areas with and without radiation, and with and without the application of tumour filtrate, were made with the view of discovering why the radiated areas were non-receptive. The following is a brief description of the appearances found:

(1) *Histology of scarification.*—(*a*) 48 hours after scarification there is a crust of dry serum containing polymorphs which replaces the epidermis: the connective tissue beneath is almost devoid of collagen and is open-meshed; cells are numerous, consisting of polymorphs, degenerated red cells, lymphocytes, monocytes, fibroblasts, some underlying mitosis; endothelial cells of the capillaries are also seen to be dividing. (*b*) 3 days after scarification: Polymorphs are now confined to the crust, fibroblasts very numerous and dividing, collagen is absent, and around the larger blood-vessels there is round-celled infiltration; endothelial cells are dividing and new blood-vessels being formed. (*c*) 4 days after scarification: The connective tissue is closely packed with fibroblasts with collagen reappearing between them, epidermis growing out under the crust of dry serum; dividing fibroblasts rarely seen; new capillaries now filled with blood. (*d*) 7 days after scarification: Epidermis now intact, collagen returned, fibroblasts lying as narrow cells between the collagen and none seen dividing; capillaries filled with blood; very little round-cell infiltration.

(2) *Histology of scarification when tumour filtrate has been applied.*—(*a*) 2 days after scarification: Indistinguishable from simple scarification except polymorphs rather more numerous. (*b*) 3 days after scarification: Resemble simple scarification, but here and there just beneath the epidermis are areas where the fibroblasts are separated one from the other by clear intercellular material without collagen; here the fibroblasts appear to be larger and dividing cells are numerous. (*c*) 4 days after scarification: Just

below the epidermis and in discrete areas, loosely packed fibroblasts contrast strongly with the normal closely packed arrangement; these cells are larger than normal, with more cytoplasm, and dividing cells are numerous. (*d*) 7 days after scarification: Small Rous tumours composed of widely dispersed spindle-cells are seen lying in a clear, structureless, intercellular substance.

From the above appearances it is concluded that the Rous tumour arises from normal fibroblasts lying in the dermis regenerating after scarification.

Turning now to radiated areas, no differences were seen whether tumour emulsion was applied or not; as tumours do not appear in radiated areas this was to be expected.

Comparing scarified radiated areas with scarified unradiated areas great differences are seen. The connective tissue in the radiated cases shows no increase in the number of cells; a few polymorphs make an early appearance and degenerated red cells occur, but there is no increase in fibroblasts; the original connective-tissue cells remain, and present degeneration with either vacuolation or pyknosis of the nucleus and shrunken cytoplasm; none are dividing. The capillaries are filled with degenerated blood and the endothelial cells are swollen and not dividing. Deep down in the dermis, not near where the Rous tumours appear close to the epidermis, round-celled infiltration is seen; here also healthy fibroblasts occur.

From the above it is concluded that Rous tumours do not appear in radiated areas because there are no healthy fibroblasts under the epidermis from which a tumour can originate.

In the previous paper it was recorded that chickens in which tumours had been made to disappear by radiation were often subsequently found to be resistant to scarification, and also to inoculation of cell-free tumour emulsions and even to the inoculation of tumour-cells. This resistance was by no means invariably observed; sometimes birds in which tumours had been made to disappear by radiation grew tumours on being subsequently scarified, or grew tumours on inoculation; sometimes, also, though they might be negative to a first inoculation, a second inoculation resulted in a tumour. This condition of resistance appears therefore to be very variable; no doubt this is largely due to want of precision in dosage, as there is no reliable method of measuring dosage as, for instance, is possible in bacteriology by counting the number of organisms used. In spite of this, it seemed desirable to obtain further information, since this resistance may also explain why tumours radiated *in vivo* disappear and do not spread to other tissues of the chicken. Search was therefore made for evidence of an anti-Rous property in the serum of resistant birds.

In the first place the effect of adding normal serum to tumour filtrate (T.F.) was studied.

Minced tumour was ground with sterile silica made up to 1 in 20 with 0·9% NaCl solution; this was spun at low speed and then passed through a Seitz clarifying filter. Blood-serum from a normal chicken was taken. The following solutions were prepared and inoculated (1 c.c.) into six chickens (both breasts) after incubation at 37°C. for 2 hours:

With Serum.

							Dilution of T.F.	Result of inoculation.
19 of 1/20	T.F.	+	1 of serum	.	.	.	= 1/20	+
10 of 1/20	„	+	9 of saline	+ 1 of serum		.	= 1/40	+
20 of 1/40	„	+ 19	„	+ 1	„	.	= 1/80	+
20 of 1/80	„	+ 19	„	+ 1	„	.	= 1/160	+
20 of 1/160	„	+ 19	„	+ 1	„	.	= 1/320	+
20 of 1/320	„	+ 19	„	+ 1	„	.	= 1/640	+

Without Serum.

19 of 1/20	T.F.	+	1 of saline	.	.	.	= 1/20	+
10 of 1/20	„	+ 10	„	.	.	.	= 1/40	+
10 of 1/40	„	+ 10	„	.	.	.	= 1/80	+
10 of 1/80	„	+ 10	„	.	.	.	= 1/160	−
10 of 1/160	„	+ 10	„	.	.	.	= 1/320	−
10 of 1/320	„	+ 10	„	.	.	.	= 1/640	−

The results show that normal serum prevents the destruction of the power to produce a tumour which occurs when T.F. is incubated at 37°C. for 2 hours. This experiment was repeated twice with similar results. The result was anticipated because during the comparison of immune and normal serum, as will be seen, it was sometimes found that tumours resulted only from mixtures containing the largest amount of normal serum, and that whereas tumours resulted from the mixture of serum with T.F. the inoculation of incubated T.F. without serum was often negative.

The action of normal serum is possibly due to an alteration of pH towards the alkaline side, which observations show does occur when normal serum is added to tumour filtrate. Baker and McIntosh (1927) have shown that alteration in pH to the alkaline side greatly increases the infectivity of tumour filtrates.

The following experiments comparing immune and normal serum have been carried out. The resistant chicken first used had the following history: Scarified on both breasts on 15.9.26. Tumours resulted and were radiated on 27.9.26. By 28.10.26 they had disappeared and the bird was re-scarified with negative result. On 11.11.26 small pieces of tumour were inoculated into both breasts with negative result: on 10.1.27 ¼ c.c. of minced tumour was inoculated. No tumour was observed until 15.2.27. On 12.4.27 the bird was killed, and a single large tumour was found on the right side with no tumours elsewhere. Blood was taken on 2.2.27 when it was presumed that the bird was immune, and on 16.2.27 when it was known to have a slow-growing tumour.

On 2.2.27 T.F. was made as previously described, the dilution being 1 in 20: the following solutions were made and incubated at 37°C. for 2 hours, these were used for scarification of one bird and inoculation of 0·5 c.c. into

another with results seen in the table. (Note: Incubated T.F. without serum did not grow.)

Dilution of serum.		Scarification (mm.).	Inoculation.
...	T.F. alone not incubated	+	+
...	10 T.F. + 10 saline ...	−	−
1/5	„ + 6 „ + 4 normal serum	40 × 15	+
1/5	„ + 6 „ + 4 immune „	0	−
1/10	„ + 8 „ + 2 normal „	24 × 20	+
1/10	„ + 8 „ + 2 immune „	4 × 4	−
1/20	„ + 9 „ + 1 normal „	25 × 24	+
1/20	„ + 9 „ + 1 immune „	6 × 6	−

On 16 . 2 . 27 a similar experiment was carried out as shown below:

Dilution of serum.	Inoculation of 1 c.c. (in mm.).	Dilution of serum.	Inoculation of 1 c.c. (in mm.).
1/40 normal	35 × 24	1/160 normal	−
1/40 immune	3 × 3	1/160 immune	−
1/80 normal	10 × 10	1/320 normal	−
1/80 immune	−	1/320 immune	−

(Note: only mixtures containing much normal serum grew tumours.)

The second immune bird had the following history: It had been radiated at six places, and on 12 . 2 . 27 scarified on these areas as well as on one normal area. Tumours appeared on five of these areas, and on 12 . 9 . 27 had grown to about 15 × 10 mm.; but afterwards they regressed and continued to do so until 29 . 9 . 27, when blood was withdrawn. After the withdrawal of blood, as has been several times noticed, the tumours again progressed, and the bird died on 10 . 10 . 27 with large tumours.

The experimental details were as before; 1 c.c. was inoculated:

Dilution of serum.		Result of inoculation.
1/5	10 of T.F. + 6 saline + 4 normal serum	+
1/5	10 „ + 6 „ + 4 immune „	−
1/10	10 „ + 8 „ + 2 normal „	+
1/10	10 „ + 8 „ + 2 immune „	−
1/20	10 „ + 9 „ + 1 normal „	+
1/20	10 „ + 9 „ + 1 immune „	−
1/40	20 „ + 19 „ + 1 normal „	+
1/40	20 „ + 19 „ + 1 immune „	−
...	10 „ + 10 „ ...	−

Note that here again there was a negative in the absence of normal serum. The next resistant fowl was scarified both sides on 26 . 7 . 27. Tumours resulted which were radiated on 4 . 8 . 27 and disappeared by 15 . 8 . 27. On 26 . 8 . 27 it was scarified both sides; on one side a nodule appeared, but had disappeared by 15 . 9 . 27. The bird was scarified again on 9 . 9 . 27, with negative result; on 29 . 9 . 27, 2·5 c.c. of minced tumour was inoculated into the right breast with negative results. Blood taken on 13 . 10 . 27.

In this experiment the tumour emulsion was spun but not filtered. The following dilutions were inoculated as before:

Dilution of serum.			Result of inoculation (mm.).
1/5	.	Normal serum .	78 × 25
1/5	.	Immune „ .	26 × 13
1/10	.	Normal „ .	75 × 31
1/10	.	Immune „ .	40 × 13
1/20	.	Normal „ .	30 × 24
1/20	.	Immune „ .	35 × 20
1/40	.	Normal „ .	80 × 24
1/40	.	Immune „ .	21 × 13
...	.	Without „ .	32 × 19

It is seen that the immune serum has not sufficient power to destroy completely the concentrated emulsion used; in this case the emulsion was spun but not filtered.

Blood was again taken from the same resistant bird on 25 . 10 . 27. At this time a tumour had appeared and was growing. The bird was killed on 18 . 11 . 27, when the tumour measured 105 × 60 mm. (Note: after withdrawing blood from an immune bird it has been several times noticed that tumours appear when the bird has been previously inoculated—as in this case.)

A spun and clarified emulsion of tumour was used. The following were the results:

Dilution of serum.	Result.	Dilution of serum.	Result.
1/5 N. .	25 × 11 mm. . .	1/20 N. .	Could not measure; tumour involved shoulder-joint.
1/5 I. .	6 × 5 „ . .	1/20 I. .	8 × 6 mm.
1/10 N. .	70 × 26 „ . .	1/40 N. .	21 × 10 „
1/10 I. .	negative . .	1/40 I. .	90 × 55 „

Tumour filtrate alone negative.

In this experiment the adverse influence of the immune serum was slight, but the fact that the resistant bird was then growing a tumour showed that the resistant condition was passing off, and a strong immune reaction could not be expected.

Experiments were next made to see whether immune serum would influence the growth of the tumour when inoculated at the same time as the tumour filtrate but into a distant part of the bird.

A very resistant bird was used which had been twice inoculated with minced tumour, the last time on 27 . 10 . 27, 1 c.c. into both breasts. Serum was taken on 3 . 11 . 27. A spun and clarified filtrate was used: 0·5 c.c. was inoculated into the left breasts of six birds. Into the right breasts was inoculated: Bird A, 1 c.c. normal serum; B, ¼ c.c. normal serum; C, 1 c.c. immune serum; D, ¼ c.c. immune serum; E and F, no inoculation. The following tumours resulted on 5 . 12 . 27: A, 79 × 33; B, 81 × 25; C, 16 × 11; D, 115 × 41; E, 33 × 22; F, 34 × 22. This experiment was repeated on 29 . 11 . 27, and gave—A, 32 × 12; B, 54 × 33; C, 32 × 17; D, 47 × 19. Thus there was no evidence of any effect.

Attention has already been drawn to the fact that mixtures of normal serum + tumour filtrate will grow whilst filtrate alone is negative.

In order to rule out the possibility that this effect is accounting for the difference observed between immune and normal serum, the following mixtures were made, incubated as before and inoculated into chickens:

Tumour filtrate was prepared as previously. Immune serum was taken from the bird used in the last experiment. Normal serum was taken from two normal birds, A and B. A mixture (X) was made of tumour emulsion 20 pts., saline 12 pts., and serum from bird A, 8 pts. The following dilutions were then made and incubated for 2 hours at 37°C.:

1/5	.	20 pts.	(X)	+ 12 pts.	saline	+ 8 pts.	immune	serum		
1/5	.	20 ,,		+ 12 ,,	,,	+ 8 ,,	normal	,,		(Bird B)
1/10	.	20 ,,		+ 16 ,,	,,	+ 4 ,,	immune	,,		
1/10	.	20 ,,		+ 16 ,,	,,	+ 4 ,,	normal	,,		(B)
1/20	.	20 ,,		+ 18 ,,	,,	+ 2 ,,	immune	,,		
1/20	.	20 ,,		+ 18 ,,	,,	+ 2 ,,	normal	,,		(B)
1/40	.	20 ,,		+ 19 ,,	,,	+ 1 ,,	immune	,,		
1/40	.	20 ,,		+ 19 ,,	,,	+ 1 ,,	normal	,,		(B)
		20 ,,		+ 20 ,,	,,					

1 c.c. of these dilutions was inoculated into two birds, the normal serum dilution into one bird, the immune serum dilutions into the other, and each bird was also inoculated with 0·5 c.c. of the tumour filtrate mixture. In 20 days the birds were killed, and the tumours shown in the table were found:

Bird $\frac{804}{22}$.				Bird $\frac{804}{23}$.			
Dilution of serum.			Result.	Dilution of serum.			Result.
1/5	N.	.	74 × 20 mm.	1/5	I.	.	*Nil.*
1/10	N.	.	70 × 15 ,,	1/10	I.	.	17 × 7 mm.
1/20	N.	.	86 × 25 ,,	1/20	I.	.	10 × 6 ,,
1/40	N.	.	41 × 23 ,,	1/40	I.	.	48 × 20 ,,
	T.N.	.	76 × 21 ,,		T.N.	.	66 × 20 ,,

This shows that the antagonistic action of immune compared with normal serum cannot be due to absence of some stimulating body present in the normal serum and absent from the immune, but must be due to some destructive property of the immune serum.

GENERAL CONCLUSIONS.

The above results show that when a Rous tumour is radiated *in vivo* with a dose of radiation more than sufficient to cause it to disappear, its power to infect by a cell-free filtrate is not destroyed. The question then arises, Why does not a tumour so radiated infect the surrounding tissues and thus produce an extension of the original tumour? The answer is that the surrounding tissues have been so altered by the radiation that a tumour cannot subsequently be produced by a cell-free filtrate. Histological examination of grafts suggests that this immutability of radiated tissue is due to the degeneration and destruction of the fibroblasts so that none are available to undergo the cancerous change produced by cell-free filtrates, and thus a tumour does not result.

In some cases birds in which tumours have been made to disappear by radiation develop secondary tumours in distant parts of the body presumably due to a transference of the infective agent from the radiated tumour. In other birds this secondary and distant infection does not occur. An explanation has been sought for this. It was found that the birds which do not grow tumours are resistant to subsequent inoculations, and that the serum of these reinoculated and resistant birds contains a property which can destroy *in vitro* the power of cell-free filtrates to produce a tumour, whereas in normal serum this is absent.

The radium used in these researches was on loan from the Medical Research Council.

This research was carried out under a grant of the British Empire Cancer Campaign.

REFERENCES.

Baker, S. L., and McIntosh, J.—(1927) *Brit. J. Exper. Pathol.*, **8**, 257.
Mottram, J. C.—(1926) *Lancet*, **2**, 1266.

FILTER-PASSING ANAEROBES IN THE UPPER RESPIRATORY TRACT.

LAWRENCE P. GARROD, M.B., M.R.C.P.

From the Department of Pathology, St. Bartholomew's Hospital, London.

Received for publication April 28th, 1928.

THE discovery by Olitzky and Gates (1920) in cases of influenza of an organism which they named *Bacterium pneumosintes*, and its cultivation (1921) in anaerobic media, has brought to light the existence of a group of allied organisms. These have been described by Olitzky and Gates (1922), Olitzky and McCartney (1923), and more recently by Mills, Shibley and Dochez (1928). These organisms are recoverable from filtrates of nasal secretion not only in catarrhal conditions but from supposedly normal subjects; they are strict anaerobes, grow as a rule sparsely in a variety of fluid and on some solid media, are all Gram-negative, and are divisible into certain groups according to their microscopic appearance, the shape and size of their colonies, and (less distinctly) by fermentation and agglutination tests.

The purpose of this paper is to record an unsuccessful attempt to cultivate *B. pneumosintes* during the 1927 epidemic, and a series of endeavours to isolate other bacteria of this group, at first with negative results and later with success. The results yield little new information beyond the fact that bacteria of this class exist in this country as well as in America, but they afford indications as to the technique which will lead to the successful cultivation of these organisms. Much further work is necessary before they can be strictly classified.

AN ATTEMPT AT ISOLATING *B. PNEUMOSINTES* FROM CASES OF INFLUENZA.

This work was undertaken in collaboration with R. G. Canti and T. Meyrick Thomas during the influenza epidemic occurring in London in January and February, 1927. In all, 15 specimens of material were examined, of which 11 were nasal washings, 1 lung juice from a fatal case of influenzal pneumonia, and 3 pleural fluids from cases of post-influenzal pleural effusion.

The 11 specimens of nasal washings were from typical cases of influenza, obtained as early as possible after the onset of the disease (9 on the first day,

2 during the course of the second). The "washings" were obtained by irrigation of the nose with warm sterile dextrose Ringer's solution, introduced through the nostril by a 20 c.c. syringe with a Pollitzer nozzle, and collected in a sterile kidney dish held below the mouth. These washings were shaken with beads in a stoppered bottle to disintegrate the mucus contained in them. Filtration and cultivation were carried out without a delay in any case exceeding 2 hours.

Cultural Methods.

(A) *Direct from material.*—It has been customary to make cultures direct from the washings in order to determine the ordinary types of flora present. This seemed a wholly unsatisfactory proceeding in that the large numbers of mouth bacteria inevitably present confused the picture. A much more satisfactory method is the cultivation on blood-agar plates of a preliminary post-nasal swab; this method was employed for the last 4 of the series of 11 cases, and gave pure or nearly pure growths of the following organisms: Meningococcus—*Streptococcus pyogenes* together with a non-hæmolytic streptococcus; pneumococcus—*Streptococcus pyogenes* together with a pneumococcus, whereas similar cultures from washings in the same cases were so crowded with mouth bacteria that it was impossible to deduce the predominance of any particular type. In the case in which a meningococcus was grown from a post-nasal swab, this organism failed altogether to appear in cultures from washings.

(B) *From filtrates.*—The emulsified washings were passed through a filter (Berkefeld V filters being used in 9 cases, other types in the remaining 2) with a negative pressure not exceeding 10 cm. Hg. The filtrate was sown in the following media:

1. Rabbit's blood glucose-agar plates (aerobic).
2. „ „ „ „ (anaerobic).
3. Smith-Noguchi medium.

Of these cultures the first (aerobic controls) were invariably sterile, and the second, which were incubated for one week in a McIntosh and Fildes anaerobic jar with the object of determining the presence of any filter-passing bacteria other than *B. pneumosintes*, failed in every case to show any such organism.

For the isolation of *B. pneumosintes*, reliance was placed on cultivation of the filtrate in Smith-Noguchi medium. About 4 drops of filtrate were sown by means of a long capillary pipette on to a piece of sterile rabbit's kidney in a Noguchi tube (200 × 14 mm.), about 10 c.c. of sterile human ascites fluid (of suitable pH and free from bile) was added, and the culture sealed by pipetting in about 1 cm. depth of vaseline. The numbers of such cultures made in each case were usually 3 from the filtrate and 3 controls. These were incubated for one week.

The appearance of a haze above the kidney in these cultures and the staining of preparations from them were not accepted as reliable indications

of growth, and it was hoped eventually to demonstrate the presence of *B. pneumosintes* by obtaining colonies on anaerobic rabbit's blood glucose-agar plates. When no such growth was obtained in subculture from the original tube, and since it is stated by Gates (1926) that the isolation of *B. pneumosintes* in plate culture, with the formation of visible colonies, may demand as many as 5 preliminary subcultivations in Smith-Noguchi medium, such subcultivations were carried out in every case. That is to say, 5 subcultures, making with the primary culture 6 in all, each occupying one week, were carried through before the final plating. The incidence of contamination in these cultures was small; no contamination due to the kidney ever occurred, and no contamination occurred in the primary cultures. The only serious trouble was due to one of a number of specimens of ascites fluid used, when a whole series of subcultures proved to be contaminated from this cause; fresh cultures were made from the series previous to it. When occasionally a single tube proved contaminated owing to faulty technique, another was duplicated to take its place.

Results and Discussion.

The results of this work are summarized in the statement that neither *B. pneumosintes* nor any other filter-passing organism was identified in any cultures made. One explanation for this might be that *B. pneumosintes* was not responsible for this outbreak of influenza, and this was the view taken at the time, but in the light of further work shortly to be described it is not improbable that the organism may have been there, but that the methods employed failed to promote its growth. The method of choice for the identification and isolation of *B. pneumosintes* is unquestionably the intratracheal inoculation of rabbits with untreated nasal washings, passage to further rabbits by inoculation with lung preparations, and eventual cultivation in Smith-Noguchi medium from this source. Thus Olitzky and Gates (1922), in the New York outbreak of 1922, examined material from 9 cases, isolating *B. pneumosintes* from 4 of them, in 3 by rabbit inoculation and passage, and only in 1 by direct cultivation. On the other hand, Wilson (1927) failed to produce typical lesions in rabbits by the intratracheal inoculation of influenza material during the English 1927 outbreak. In any further investigations of the ætiology of this disease, it would appear desirable to employ both cultural methods and rabbit passage in an adequate series of cases.

A STUDY OF THE FILTER-PASSING ANAEROBES (OTHER THAN *B. PNEUMOSINTES*) IN THE UPPER RESPIRATORY TRACT.

No further influenza material having been available, the writer has attempted to cultivate the filtrable micro-organisms other than *B. pneumosintes* which have been described as existing in the upper respiratory tract.

The experiments concerned have been divisible into two series. In the first, the technique of Olitzky and Gates (1922) was followed. The material (7 specimens of nasal secretion or washings from cases of common cold, 1 of saliva and 3 of adenoid tissue) was emulsified in saline or Ringer's solution,

and passed through a Berkefeld V filter under a negative pressure of 10 cm. Hg. The filtrate was sown on 5% rabbit's blood glucose-agar plates, aerobic and anaerobic (the latter in a McIntosh and Fildes jar), and in Smith Noguchi medium, which was subsequently plated as above. All cultures were incubated for one week. No filter-passing micro-organism of any type was isolated by these methods.

Since these methods serve the purpose of studying the bacteriology of the common cold, it is of some interest to note the results of direct cultivation of the material. As already observed, the direct cultivation of washings, although an accepted feature of such investigations, is highly unsatisfactory, owing to the predominance in this material of mouth bacteria. Cultures on blood-agar plates were therefore made from the nasal secretion itself or post-nasal swabs, or both. In 11 out of a total of 16 cases of common cold investigated during the course of this work, a pure or nearly pure growth was obtained from these sources of one of the organisms commonly recognized as capable of producing acute catarrh. These were in 7 cases pneumococci, and in 1 each *B. influenzæ*, *B. coryzæ segmentosus*, *M. catarrhalis* and *Streptococcus pyogenes*. In the remaining 5 cases, either very little or a mixed growth was obtained.

A second series of cases has been investigated by the methods employed by Mills, Shibly and Dochez (1928). The important difference in their technique is the substitution of buffered broth for dextrose Ringer's solution for the purpose of nasal washing, or the emulsification of pure nasal secretion. In addition, larger quantities of the filtrate (about 0·5 c.c.) were sown on plates, and anaerobic cultures were also made in *B. coli* broth and rabbit's blood broth; the use of Smith-Noguchi medium was discontinued. Ten specimens of material have been examined by these methods, of which 3 were nasal secretions or nasal washings (with broth) from cases of common cold, 5 nasal washings from normal subjects, 1 adenoid material and 1 fæces. In all these specimens, with the exception of 1 nasal secretion from a common cold and the 1 adenoid material, one or more types of filter-passing anaerobic micro-organism have been found. Of these the majority appeared in primary culture on rabbit's blood-agar or rabbit's blood glucose agar plates (the addition of glucose to this medium appears to be unnecessary). Nine strains were isolated by culture of the filtrate direct to this medium, 5 by primary culture in rabbit's blood broth, and 2 by primary culture in *B. coli* broth, both these types of culture being subsequently plated.

The main characters of these organisms are as follows: Their colonies are transparent and small, their shape only being distinguishable as a rule with the low power of a microscope. By their shape they are divisible into two groups: a uniform type, invariably forming a hemispherical colony with a clear-cut margin; and a pleomorphic type, in which colonies of the same strain may in different cultures be flat, slightly raised or markedly conical, with either a smooth or a crenated edge. The former type in stained films is a coccus occurring in hazy masses, the individual cocci being about one-fifth of the diameter of a staphylococcus, and therefore on the border-line of resolution with the $\frac{1}{12}$″ objective. One strain of this type (the only one so tested) has been found to be re-filtrable after two generations of subculture. The second type is a bacillus, always thin, usually shorter than *B. influenzæ*, sometimes

curved; repeated subculture leads to an increase in length. All strains isolated have been Gram-negative. None are capable of growth except under anaerobic conditions, but cultures have been found to survive exposed to the air at room temperature for periods varying from 2 to 6 days. Some have been found to multiply and others apparently not, in Smith-Noguchi medium. Attempts to obtain mass cultures in flasks of *B. coli* broth, in order to produce emulsions for agglutination, have been unsuccessful. The alternatives are the use of large numbers of plates, or some suitable fluid medium which has yet to be found; broth containing a small proportion of haemolysed and filtered rabbit's blood is now being tried. Until suitable emulsions for agglutination and other tests can be prepared, no further progress is likely to be made in classification.

Discussion.

Two comparatively slight changes in technique led to the successful cultivation of these organisms, viz. the substitution of buffered broth for dextrose Ringer's solution as a diluting and emulsifying agent, and the inoculation of a larger amount of the filtrate on plates. (That this latter point is of importance is shown by the fact that when different quantities are sown on several plates, growth is sometimes obtained from, for example, 8 drops, and not from 4. It appears that a very moist surface is necessary for primary growth.)

These experiments demonstrate the existence in the upper air-passages of a group of organisms, the distinguishing characters of which are at present imperfectly defined. The possibility that they may have a much wider distribution in the human body is suggested by the discovery of a similar organism in a specimen of fæces.

SUMMARY.

1. An attempt to isolate *B. pneumosintes* from material from 15 cases of influenza by cultural method was unsuccessful.

2. The employment of similar methods failed to demonstrate the presence of any other filter-passing micro-organisms in these and other specimens of material.

3. The use of modified methods has demonstrated the presence of filter-passing anaerobic micro-organisms in a high proportion of specimens of material from the upper respiratory tract, from both healthy and diseased subjects.

These organisms form a group which awaits more exact classification, and identification as to pathogenicity, if any.

The research described in this paper was assisted by a grant from the Medical Research Council, to whom my thanks are due. I am also indebted to Dr. R. G. Canti for valuable help and advice.

DESCRIPTION OF FIGURES.

FIG. 1.—Photograph of colonies of the coccoid type of naso-pharyngeal filter-passer. Six days' growth on anaerobic rabbit's blood agar. × 12 diameters.

FIG. 2.—Colonies of a bacillary type of nasopharyngeal filter-passer, showing both conical and flat forms. Six days' growth on anaerobic rabbit's blood agar. × 12 diameters.

FIG. 3 —Colonies of another strain of the bacillary type. Same conditions of growth and magnification.

REFERENCES.

GATES, F. L.—(1926) *J. Exp. Med.*, **44**, 787.
MILLS, K. C., SHIBLEY, G. S., AND DOCHEZ, A. R.—(1928) *Ibid.*, **47**, 1923.
OLITZKY, P. K., AND GATES, F. L.—(1920) *J. Amer. Med. Assoc.*, **74**, 1497.—(1921) *J. Exp. Med.*, **33**, 713.—(1922) *Ibid.*, **36**, 501.
OLITZKY, P. K., AND MCCARTNEY, J. E.—(1923) *Ibid.*, **38**, 427.
WILSON, G. S.—(1927) *Lancet*, **1**, 1123.

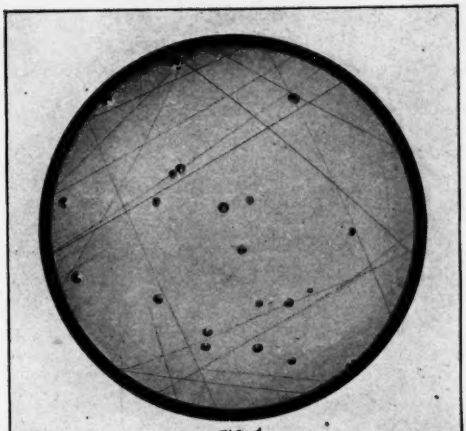


FIG. 1.

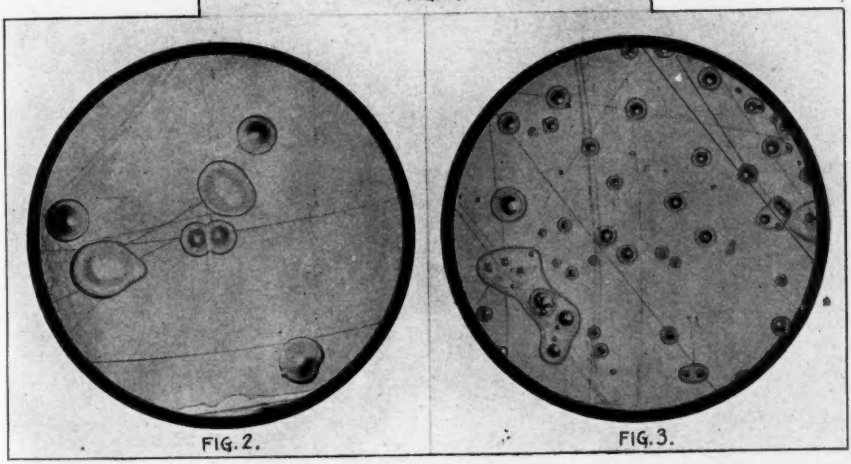


FIG. 2.

FIG. 3.

Garrod.

UNIV OF MICH.

BAIRD & TATLOCK (LONDON) LTD

An Improved Form of

ANAËROBIC JAR

By Drs. McINTOSH & FILDES.

(*Reference: 'British Journal of Experimental Pathology,' 1921, II, 153.*)

B. 1488. **Price £3 15s. each**

Sole Address:

14-15 CROSS STREET HATTON GARDEN LONDON E.C.1

MICROSCOPIC STAINS AND SOLUTIONS MOUNTING MEDIA

PREPARATIONS for all Scientific Purposes.

STANDARD QUALITY.

Price List on application.
Telephone: PUTNEY 1468.

Manufactured by

GEORGE T. GURR

136 NEW KING'S ROAD, FULHAM, LONDON, S.W. 6.

MICROSCOPIC STAINS

Specially made for Bacteriological and Histological Work.

The B.D.H. Standard Stains are tested histologically before they are issued. They can be relied upon to give the correct reaction for each stain.

Applications are invited for a copy of our Price List and Booklet upon Clinical Pathology and the use of Stains.

THE BRITISH DRUG HOUSES, LTD.

Makers of Fine Chemicals,

Graham Street, City Road, London, N. 1.

Chemical Works: Wharf Road, London, N. 1.

THE BRITISH JOURNAL OF EXPERIMENTAL PATHOLOGY.

VOL. IX, No. 3. JUNE, 1928.

CONTENTS.

ROBERTS, W. MORRELL.—OBSERVATIONS ON THE NATURE OF THE VAN DEN BERGH REACTION 107

NEWMAN, C. E.—BILIRUBIN AND THE VAN DEN BERGH REACTION . . 112

MAGEE, H. E., ANDERSON, W., AND GLENNIE, AGNES E.—SOME OBSERVATIONS ON THE EFFECTS OF ETHER ANÆSTHESIA ON THE COMPOSITION OF THE BLOOD AND THEIR RELATION TO THYROID FUNCTION 119

HILL, LEONARD, AND MCQUEEN, JAMES M.—THE PRINCIPLES OF CAPILLARY BLOOD-PRESSURE MEASUREMENTS AS APPLIED TO GLOMERULAR AND TUBULAR FUNCTION IN THE KIDNEY 127

HILL, LEONARD.—THE CAPILLARY BLOOD-PRESSURE IN RELATION TO ŒDEMA AND CEREBRAL AND INTRA-OCULAR PRESSURE 135

MOTTRAM, J. C.—THE EFFECT OF RADIATION ON ROUS CHICKEN TUMOUR . 147

GARROD, LAWRENCE P.—FILTER-PASSING ANAEROBES IN THE UPPER RESPIRATORY TRACT 155

Subscriptions and Business Correspondence should be addressed to the Publishers, 28, Gower Place, London, W.C. 1. The JOURNAL is only sold *at the subscription rate* by the year or volume. General correspondence for the Editors should be addressed to Dr. P. FILDES, London Hospital, E. 1, but letters relating to biochemical and physiological papers to Professor E. C. DODDS, Middlesex Hospital, W. 1.

GRAHAM SCHOLARSHIP IN PATHOLOGY.

THE Senate of the University of London invite applications for the Graham Scholarship in Pathology, value £300 per annum, in the first instance for two years, founded under the will of the late Dr. Charles Graham to enable "a young man to continue his pathological researches and at the same time to secure his services to the School of Advanced Medical Studies connected with the University College Hospital as a Teacher under the direction of the Professor of Pathology."

The successful candidate will, if possible, take up the Scholarship as from the 1st October, 1928. The Scholarship is in no way restricted to students of Medical Schools of the University. Applications must be made to the undersigned before 27th June, 1928. Further particulars should be obtained from the Academic Registrar, University of London, South Kensington, S.W.7.

FRANKLIN SIBLY, *Principal Officer.*

PRINTED BY ADLARD AND SON, LIMITED, LONDON AND DORKING.